PLANT TOXICITY TESTING WITH SEDIMENT AND MARSH SOILS

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EXECUTIVE SUMMARY

Contaminated sediments are a major problem in many ponds, lakes, and marshes of the United States. Besides creating problems *in situ*, they are significant non-point sources for contamination downstream.

The National Park Service is aware of potential dangers of contaminated sediments to water resources and supports efforts to detect and mitigate those dangers. This report, which describes principles and methods for laboratory detection of potential effects of contaminated sediments on aquatic plants, may be used by park personnel wherever sediment contamination is suspected. The report was prepared as supplementary reading for a course entitled "Soil and Plant Toxicity Assessment" given at the 12th Annual Meeting of the Society of Environmental Toxicology and Chemistry, Seattle, WA, on November 3, 1991. It should be of value to those who are interested in environmental toxicology.

ABSTRACT

A short account of the principles and practices of toxicity testing with aquatic plants and sediments is given. Aquatic (wetland, marsh) plants have been shown to be sensitive to toxicants in natural and synthetic sediments, and advantages and disadvantages of each type of sediment in toxicity testing are described. Toxicological studies with *Echinochloa crusgalli*, *Sesbania macrocarpa*, and *Spartina alterniflora* are described, but other experimental species need to be adapted for use in impact analysis and risk assessment. It is concluded, after comparison of results from seed germination, hydroponic, and sediment tests, that the latter best simulate the unique field conditions under which plants are exposed to pollutants.

INTRODUCTION

Submerged and emergent vascular plants are dominant features of wetlands and, in association with sediments, determine the structure and function of these important ecosystems. In fact, wetlands have been defined in relation to their sediments as "areas that are inundated or saturated by surface- or ground-water, at such a frequency and duration that under natural conditions they support organisms adapted to poorly aerated and/or saturated soil" (Lugo, 1990). Wetland (aquatic) plants are adapted to the conditions of such sediments, and risk analyses of probable effects of toxicants on them must be conducted under the unique aquatic plant/sediment system.

Sediments are major sinks or reservoirs for pollutants. Pesticides, other toxic substances, and nutrients adsorb to organic and inorganic particles in water and are deposited with them in sedimentary areas such as lakes, ponds, and freshwater and saltwater marshes. Because sediments act as reservoirs, toxic effects may be felt long after the original pollution event is past. When in place in aquatic ecosystems, toxic sediments serve as major non-point sources of pollution to the benthos, water column, or downstream areas by redeposition and erosion. Toxic sediments are now recognized as a major pollution problem, and new methods are rapidly being developed for their analysis with algae, plants, and animals. The American Society for Testing and Materials (ASTM) supports a large subcommittee for development of ecotoxicological studies on sediments (Subcommittee E47.03, "Sediment Toxicology").

This short account of toxicity testing with marsh plants and sediment is designed as an introduction to the subject. Its basic assumptions are that structure and functions of wetlands can be affected by toxicants in sediments and that laboratory tests can detect possible or probable injury to aquatic plants under specific circumstances. The methods given here may be modified or used directly for routine toxicity testing or for experimental studies in which environmental variables are manipulated.

PRINCIPLES OF SEDIMENT TOXICITY TESTING WITH VASCULAR PLANTS

Aquatic vascular plants are potentially of great value for estimation of soil and sediment toxicity. Their roots are in close contact with the particles of these substrata, and they absorb and translocate substances, including toxics, from interstitial water to other parts of the plants. Because they are in such close association, rooted aquatic vascular plants modify their substrata and are affected by them. They are also sensitive to pollutants and are thus good test subjects for identification of toxic sediments and marsh soils.

Although terrestrial vascular plants are used commonly for toxicity testing of soil, aquatic vascular plants have not been used extensively for such studies of sediment. A major text on aquatic toxicity testing (Rand and Petrocelli, 1985) mentions only microalgae (unicellular algae, phytoplankton) for use in phytotoxicity tests, and an otherwise outstanding review of aquatic toxicity testing (Munawar *et al.*, 1989) describes studies on only one species of aquatic vascular plant, *Lemna minor* (duckweed), a floating plant that does not have contact with a solid substratum. In the same publication, Ahlf *et al.*, (1989) used germination and initial growth of rye grass (*Lolium multiflorum*) and cress (*Lepidium sativum*) in phytotoxicity tests on sediments. In a review of sediment toxicity and bioaccumulation testing, Ingersoll (1991) did not mention use of vascular plants.

The fact that rooted aquatic plants are sensitive to toxicants in sediment has been demonstrated by Walsh *et al.*, (1990, 1991a, b, *in press* a,b). Copies of these reports are appended. As with other toxicity tests, the conditions under which plants are exposed to bioactive substances strongly determine response. Many environmental variables affect survival and growth of aquatic plants (Table 1). All of these can interact with pollutants and must be considered when interpreting toxicity data. Thus, structure, composition, and physical and chemical conditions of soil or sediment, inherited plant sensitivity or resistance, age and size of the plant, length of time of exposure, temperature, light intensity and quality, polarity of the toxic molecule and its degradative properties all affect response.

A brief introduction to the properties of sediments is given below, followed by a discussion on toxicity testing with plants. The term "sediment" will be used to designate the substratum upon which and within which aquatic plants grow. Aquatic plants are "those species which normally start development in water and must grow for at least a part of their life cycle in water, either completely submersed or emersed" (Muenscher, 1972).

PROPERTIES OF SEDIMENTS

Sediment is particulate matter that has been transported by wind, water, or ice, or that has been precipitated from water. Its chemical composition and physical properties are determined by its origin and how it was changed by physical, chemical, and biological processes before and after deposition in aquatic ecosystems.

Inorganic Sediment - This sediment is composed of particles classified by size (Table 2) and formed predominantly from igneous granite (mainly Si0) or sedimentary limestone (mainly $CaCO_3$). Structures of three secondary minerals derived mainly from granite are illustrated in Fig. 1. Sediment textural classes are defined by the relative amounts of sand, silt, and clay (Fig. 2).

Table 1. Aspects of environmental variables that affect survival and growth of aquatic plants. Modified from Scott (1974).

<u>Climatic</u>	
Solar radiation	Spectral composition, intensity, direction, periodicity
Terrestrial back radiation	Intensity, integration
Temperature, air	Degree, periodicity, integration, lateral and vertical variation
Temperature, sediment	Degree, periodicity, integration, lateral and vertical variation, freeze-thaw phenomena
Water, vapor	Evaporation, transpiration
Water, precipitation	Cloud, fog, dew, salt spray, rain, snow, pH
Water, soil	Content, tension, supply rate, aeration
Gasses, atmospheric	Oxygen and carbon dioxide contents, ozone, pollutant gasses
Weather phenomena	Wind, frequency, force, direction, evaporation, transpiration, abrasive agents
<u>Edaphic</u>	
Parent material	Minerals present, weathering
Physical properties	Texture, mechanical analysis, moisture, stability
Chemical properties	Clay mineralogy, organic compounds, cation exchange capacity, pH, redox, macro- and micro-nutrients, toxic substances
Biotic properties	Soil flora and fauna

Table 2. Particle size categories of geological materials. The phi value is the logarithmic transformation of particle diameter in mm based on the negative log to the base 2. (Lincoln *et al.*, 1982)

Diameter mm	Phi value		
		Boulder	
256	-8	Cobble	Gravel
64	-6	Pebble	
4	-2	Granule	
2	-1	Very Coarse	
1	0	Coarse	
0.5	1	Medium	Sand
0.25	2	Fine	
0.125	3	Very Fine	
0.0625	4	Silt	
0.0039	8	Clay	Mud
0.00024	12	Colloid	

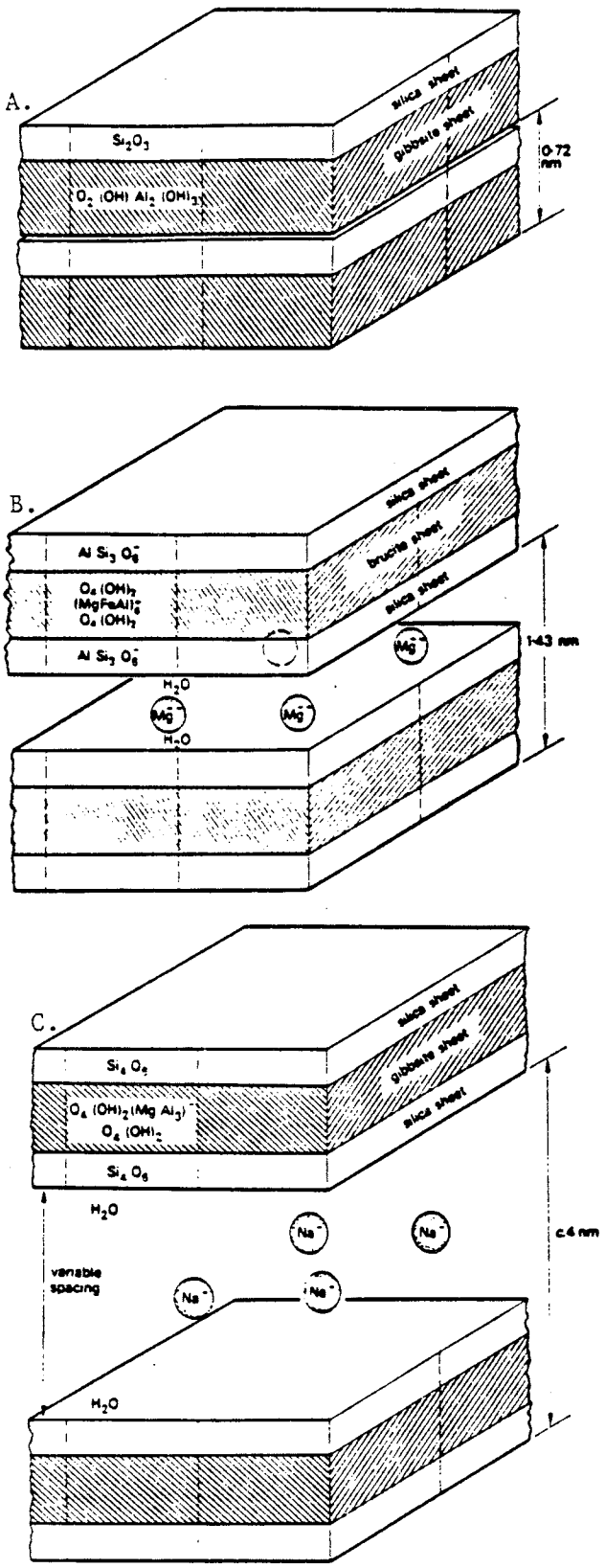

Figure 1. Structure of A. kaolinite, B. vermiculite, and C. montmorillonite. From White (1987)

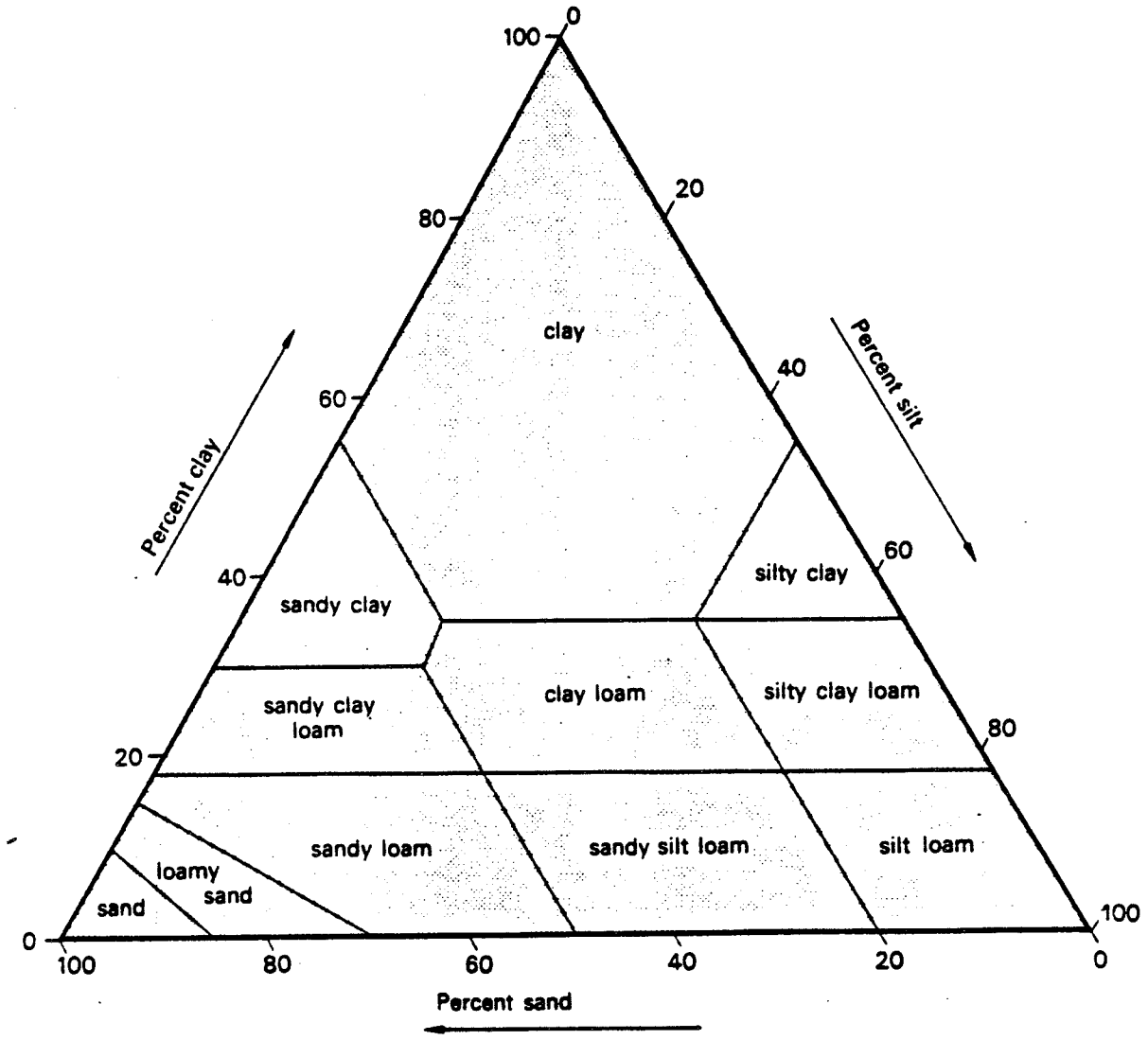

Figure 2. Soil textural classes. From White (1987) after Hodgson (1974).

Table 3. Cation exchange capacity and size of sediment particles. Black, (1968).

	Diameter of particles, mm	Surface area cm^2/g	CEC Cmols (+)/kg
Silt	0.02-0.005	1,800	3
	0.005-0.002	6,200	7
Coarse clay	0.002-0.001	16,000	22
	0.001-0.0005	30,000	35
	0.0005-0.0001	74,000	52
Fine clay	0.0001-0.00005	320,000	56
	<0.00005	920,000	63

Texture - Texture is defined as the size distribution of the particles that form the sediment. The term "structure" is often used synonymously with "texture," although technically they are different. Texture is a very important property of sediments because it controls the drainage properties, amount of pore space between particles (and thus the ease with which roots and rootlets may penetrate), volume of pore (interstitial) water, and soil temperature. Texture controls the amount of water available to plants (the amount is greater in sediments of moderately fine texture than in sediments of coarse texture), and availability of sediment nitrogen, a limiting nutrient, usually increases as texture becomes finer. This combination of availability of water and essential nutrients can strongly affect plant growth and results of toxicity tests.

Relative amounts of sand, silt, and clay may also affect the dissolved constituents of pore water. Texture is important here because dissolved substances are the only ones available for uptake by plants. Clays adsorb more dissolved organic matter than do sands or silts, so that plant response to contaminated soil of high clay content may be less than that in a similarly contaminated sandy soil.

The property of sediment most important in regulation of concentrations of dissolved constituents of pore water is the cation exchange capacity (CEC). This is defined as the total amount of exchangeable cations that soil or sediment holds, as determined by leaching with a neutral salt such as 1N KCl or 0.1N $BaCl_2$ (Russell, 1973). The CEC of the mineral fractions of soils is derived from the dissociation of cations from particle surfaces, and therefore increases with the surface area of the particles. Surface area and CEC per unit weight increase rapidly with decrease in particle size (Black, 1968; Table 3). Thus, the clay fraction is extremely important in the binding of ionic and polar substances to sediments, including the amount bound and the strength by which it is bound.

Organic Matter - The organic matter of sediments is composed of living organisms such as bacteria, fungi, algae, plant roots, protozoa, etc., the remains of dead organisms in various states of

decomposition, and exudates of living organisms (bacteria, fungi, root exudates, etc.). White (1987) suggested that non-living soil organic matter may be broadly classified as (a) macro-organic matter: plant and animal debris, (b) light fraction: partly humidified and very fine plant and animal remains, and (c) humified fraction: organic matter that has been reduced to humus. Aiken *et al.*, (1985) defined humic substances as "A general category of naturally occurring biogenic, heterogeneous organic substances that can generally be categorized as being yellow to black in color, of high molecular weight, and refractory." They described three fractions of humic substances in relation to their solubilities: humin, humic acid, and fulvic acid. These substances have been the subjects of numerous studies (Schnitzer and Khan, 1978; Tate, 1987; Kumada, 1987; Drever, 1988; Saiz-Jimenez *et al.*, 1989), and they affect toxicity of organic and inorganic substances in sediment (Suffet and MacCarthy, 1989).

Organic matter in sediment may be in the dissolved, colloidal, or particulate phases. The dissolved and colloidal portions may be free in the pore water or they may be adsorbed to clay, forming a clay-humus complex. The main products of humification are organic colloids with a high surface area, a high CEC, and the ability to chelate metal ions. These properties make organic matter extremely important in sediment toxicity, especially in relation to clay concentration. Toxicity tends to be inversely related to concentrations of clay and organic matter.

Particulate organic matter, because it can hold a weight of water greater than its own weight, can influence the water holding capacity of a sandy soil (Thompson and Troek, 1973), and thus affect the growth rates of plants by adsorption of toxicants and provision of water.

Aeration - The proper sediment atmosphere is vital to plant survival and normal growth. Sediment pores that are not filled with water contain lacunae that contain the sediment atmosphere. This atmosphere contains higher amounts of carbon dioxide and lower amounts of oxygen than the atmosphere above the sediment because of uptake of oxygen and release of carbon dioxide from metabolism by roots and soil organisms and by decomposition of organic matter. In aquatic systems, gaseous exchange between sediment and the overlying water is facilitated by bioturbation and water motion. In marshes, it is also facilitated by burrowing organisms.

Most plants require sediment atmospheric oxygen for root function, although some, such as mangroves, have anatomical devices for conduction of oxygen from the atmosphere above the sediment to the roots. It is important that the sediments of toxicity tests with plants never become anaerobic to ensure that effects on growth are due only to the toxicant.

pH - For the purpose of pH measurement, sediment is considered to be a suspension of particles in water. The pH of such a system is determined by the ionic atmosphere around the particles, i.e., the relative amounts of acidic (H^+ and Al^{+++}) and base (Ca^{++} and Mg^{++}) cations on its cation exchange sites. The pH of a sediment depends on the salt concentration in the soil solution and the carbon dioxide concentration in the sediment atmosphere (Russell, 1973).

By itself, pH has no effect on plant survival and growth. However, it does control edaphic factors that can affect plants. pH influences the solubility of plant nutrients, the amounts of nutrients stored on cation exchange sites, and the rate of weathering (Thompson and Troek, 1973). Cation exchange capacity is directly related to pH: a rise in pH produces an increase in CEC (Black, 1968).

Control of pH is extremely important in toxicity testing of sediments. Many pollutants are affected directly by pH, being more or less active, susceptible to degradation, or adsorbed to particles at various pHs. For example, at pH 4, aluminum (Al^{+++}) becomes soluble and can be strongly toxic to aquatic biota, whereas at higher pH, Al is complexed and not detrimental

Redox potential - Redox (oxidation - reduction, Eh) reactions are those in which a molecule or ion is reduced from a more oxidized state to a less oxidized state, or *vice versa*, through the transfer of electrons (White, 1987). The redox potential is a measurement, expressed in volts or millivolts (mV), of the tendency for a redox reaction to occur. High redox potential is associated with an oxidizing atmosphere, low redox potential with a reducing atmosphere. For a clear and succinct discussion of redox potential in waterlogged soil, see Russell (1973).

Since low redox potential is associated with reducing conditions (low pH and dissolved oxygen concentration, presence of hydrogen sulfide), higher potentials must be maintained to ensure healthy plants. Toxicity tests are best conducted at redox potentials of 200 mV or greater at pH between 6 and 7. This can usually be achieved easily by avoidance of anaerobic sediment conditions.

PLANT REQUIREMENTS

Texture - Individual plant species have optimal requirements for sediment texture. Texture affects the rate of growth, plant form, and function of roots, which in turn affect the well-being of the plant. It affects root penetration and branching, production of root hairs, root cellular morphology, water and nutrient uptake, the amount of photosynthate required to form and sustain roots, oxygen utilization by roots, and activities of symbiotic root bacteria and fungi (Glinski and Lipiec, 1990).

Pore space, which is related to mechanical impedance to root growth, is probably the most important attribute of sediment with regard to growth. Pore space, or porosity, is expressed as the ratio:

$$\textit{Pore Space Ratio} = \frac{\textit{volume of pores}}{\textit{total soil volume}}$$

Porosity does not indicate the size or shape of the pores, which are dependent upon the size and shape of the sediment particles, nor does it indicate the relative amounts of space occupied by water and air.

A general rule is that a pore space ratio of 0.5 m^3/m^3, or 50% pore space is desirable for most plants. Between 10 and 25% clay and approximately equal amounts of silt and sand and several percent of organic matter makes a very good soil for most uses (Thompson and Troek, 1973). However, it is best to determine the optimal texture for growth of test plants before initiation of toxicity studies.

Nutrients - Plants require an array of nutrients in specific concentrations and relative amounts for survival and growth. Insufficient quantities of nutrients limit growth, whereas quantities above those needed for optimal growth may be toxic. Carbon dioxide from the air and water from the sediment

are the sole sources of carbon and hydrogen ions for plants. Mineral nutrients include salts of nitrogen, phosphorus, potassium, sulfur, magnesium, calcium, iron, zinc, copper, manganese, boron, and molybdenum. Cobalt, silicon, and aluminum may also be necessary. Fertilizers that contain correct amounts of these nutrients are available commercially, but it is usually better to prepare liquid fertilizers from laboratory chemicals for improved quality assurance. A good fertilizer was described by Hoagland and Arnon (1950).

Numerous excellent books on plant nutrition have been published, and one should be consulted before tests with plants are begun. The fertilization regime is dependent upon the needs of the test species and whether or not the test pots are drained or closed.

Light - Light is required by plants for photosynthesis and normal growth rate and form. Too little light will result in low productivity and elongation and thinning (etiolation) of the plant axis. Too much light causes reduced productivity and damage to plant structures.

Intensity and quality of light should be carefully controlled in toxicity tests with plants. Most plants will grow normally under 300-600 $\mu E/m^2/sec$ of photosynthetically active radiation (PAR, 400-720 nm wave length) with a diel light: dark cycle determined by experimental needs. A 16h light: 8h darkness cycle is generally acceptable.

Water - Water is not a problem in toxicity tests with aquatic plants because the sediment is kept waterlogged in short-term tests. A waterlogged (flooded) sediment is one whose pore space is filled to capacity. In long-term tests, the sediment is kept moist constantly.

Temperature - Temperature of the air and sediment affect plant growth. Each species has an optimal temperature that must be maintained, within limits, in toxicity tests. Some species require diel variation in temperature for optimal growth. It is necessary, therefore, to expose plants to toxic sediments in plant growth chambers or greenhouses where temperature variation is minimal or under controlled temperature regimes.

Humidity - Transpiration rate is related indirectly to relative humidity: as humidity falls, transpiration rate increases. If the transpiration rate is too high, the plant may wilt. Toxicity tests should be conducted at a relative humidity that does not place stress upon the plant.

CHOICE OF TEST SPECIES

Only a few marsh plant species have been used for sediment toxicity testing in the laboratory, and there is a need for development of tests with new species. At present, toxicity testing is limited to species for which seeds are available. Seeds may be collected in the field and planted in uncontaminated natural or synthetic sediment in the laboratory. The plants that develop from them may be grown to maturity and their seeds harvested and replanted to build a seed source whose background is known. Otherwise, seeds may be purchased from commercial dealers in bulk and used directly in toxicity tests and as starters of laboratory cultures for seeds. Seeds and grown individuals ready for transplanting may be purchased from Wildlife Nurseries, Ashkosh, WI (freshwater) and Environmental Concern, St. Simons, MD (saltwater).

Although several species of freshwater and saltwater marshplants have been used in hydroponic uptake and toxicity studies (Lee *et al.*, 1981), I am familiar only with sediment methods for *Echinochloa crusgalli* (Linneaus) Palisot de Beauvois var. *crusgalli* and var. *zelayensis* (Poaceae) and *Sesbania macrocarpa* Muhl. ex Raf. (Leguminosae) (freshwater) and *Spartina alterniflora* Loisel (Poaceae) (saltwater). Publications that describe methods and studies with these species are described in Appendices A, B, and C.

METHODS OF TOXICITY TESTING WITH SEDIMENT/PLANT SYSTEMS

Toxicity testing with vascular marsh plants is currently in a developmental stage, and I know of no "standard" methods for such tests. Sediment/plant tests are potentially of great value for estimation of possible effects of field sediment samples and of specific toxic substances.

The only published methods for sediment/plant toxicity tests that I am aware of are given in detail in Appendices A, B, and C. Comparisons of natural and synthetic sediments are presented in Appendices D, E, and F. The methods address preparation of seeds and sediments, transplantation of seedlings to toxic sediments, length of exposure time, endpoints of toxicity tests, and statistical analyses. They are simple and adaptable for most requirements.

Synopsis of Method

1. Germinate seeds in uncontaminated sand under environmental conditions to be used in the toxicity exposure.

2. Transplant seedlings to control and contaminated soils or sediments.

3. Grow the seedlings for a predetermined period of time under carefully controlled conditions of light, temperature, and watering regime.

4. Harvest seedlings, measure height, and weigh while fresh.

5. Divide seedlings into roots, stems, and leaves; dry and weigh.

6. Apply statistical analyses to determine effects on weights of plant parts.

DISCUSSION

It is clear that the sediment/plant system is, like natural systems, very complex, and that toxicity tests must be carefully controlled. All of the sediment and plant considerations presented here can affect results.

The major concern of laboratory toxicity tests is how results aid in prediction of possible effects in natural ecosystems. Results from sediment/plant, seed germination, and hydroponic tests (Appendices A, B, and C) often differ, and it is concluded that germination and hydroponic tests are valuable for estimation of effects and uptake of dissolved substances. However, most vascular plants are exposed to toxic substances in the rooting sediment. As shown above, the complex features of sediments, and plant interactions with sediment, light, and atmospheric conditions,

greatly modify the toxic response, suggesting that germination and hydroponic tests give little, if any, information on possible field effects. Sediment tests consider the roles of sediment in plant growth and pollutant adsorption/desorption kinetics. Also, other factors that affect plant growth (water, nutrients, light, etc.) are supplied in a more field-related manner in sediment tests.

Sediment/plant tests are potentially of great value for estimation of possible effects of field samples and specific toxic substances. Papers in the appendices demonstrate that plant growth was affected by heavy metals, organic substances, and effluents in sediment, that synthetic sediments are valuable in toxicity tests, and that degree of plant response to toxicants is related to sediment composition. Also, some plant species are more sensitive to toxicants than others. Since plants are susceptible to toxicants in soils and sediments, information from most batteries of tests, e.g. the Sediment Quality Triad (Chapman, 1990), could be broadened by addition of plant tests.

LITERATURE CITED

Ahlf, W., W. Calmano, J. Erhard, and U. Forstner. 1989. Comparison of five bioassay techniques for assessing sediment-bound contaminants. Hydrobiologia 188/189: 285-289.

Aiken, G.R., D.M. McKnight, R.L. Wershaw, and P. MacCarthy. 1985. Humic Substances in Soil, Sediment, and Water: Geochemistry, Isolation, and Characterization. Wiley, New York. 692 pp.

Black, C.A. 1968. Soil-Plant Relationships, 2nd Ed. Wiley, New York. 792 pp.

Chapman, G. 1990. The sediment quality triad approach to determining pollution-induced degradation. Sci. Total Environ. 97-98: 815-825.

Drever, J.I. 1988. The Geochemistry of Natural Waters. Prentice Hall, Englewood Cliffs. 419 pp.

Glinski, J. and J. Lipiec. 1990. Soil Physical Conditions and Plant Roots. CRC Press, Inc., Boca Raton. 250 pp.

Hoagland, D.R. and D.I. Arnon. 1950. The Water Culture Method for Growing Plants Without Soil. California Experiment Station Circular 347. Berkeley, CA.

Hodgson, J.M. (ed.) 1974. Soil Survey Field Handbook. Soil Survey of England and Wales, Technical Monograph No. 5, Harpenden.

Ingersoll, C. 1991. Sediment toxicity and bioaccumulation testing. ASTM Standardization News, April. pp. 28-33.

Kumada, K. 1987. Chemistry of Soil Organic Matter. Elsevier, New York. 235 pp.

Lee, C.R., T.C. Sturgis, and M.C. Landin. 1981. Heavy metal uptake by marsh plants in hydroponic solution cultures. J. Plant Nut. 3: 139-151

Lincoln, R.J., G.A. Boxshall, and P.F. Clark. 1982. A Dictionary of Ecology, Evolution and Systematics. Cambridge Univ. Press, Cambridge 298 pp.

Lugo, A.E. and M. Brinson. 1990. Ecosystems of the World 15: Forested Wetlands. Elsevier, New York. 497 pp.

Muenscher, W.C. 1972. Aquatic Plants of the United States. Cornell Univ. Press, Ithaca. 374 pp.

Munawar, M., G. Dixon, C.I. Mayfield, T. Reynoldson, and M.H. Sadar (eds). 1989. Entire issue of Hydrobiologia (188/189), 670 pp.

Rand, G.M. and S.R. Petrocelli. 1985. Fundamentals of Aquatic Toxicology: Methods and Applications. Hemisphere Pub. Co., Washington, D.C. 666 pp.

Russell, E.W. 1973. Soil Conditions and Plant Growth, 10th ed. Longman, London. 848 pp.

Saiz-Jimenez, C., R.A. Rosell, and J. Albaiges. 1989. Advances in Humic Substances Research. Special Issue, Sci. Total Environ. 81/82: 1-723.

Schnitzer, M. and S.U. Khan. 1978. Soil Organic Matter. Elsevier, New York. 319 pp.

Scott, D. 1974. Description of relationships between plants and environment. pp. 49-69, *in* Handbook of Vegetation Science, Part IV, Vegetation and Environment, ed. by B.R. Strain and W.D. Billings. Junk, The Hague. 193 pp.

Suffet, I.H. and P. MacCarthy. 1989. Aquatic Humic Substances: Influence on Fate and Treatment of Pollutants. American Chemical Society, Washington, D.C. 864 pp.

Tate, R.L., III. 1987. Soil Organic Matter: Biological and Ecological Effects. Wiley, New York. 291 pp.

Thompson, L.M. and F.R. Troek. 1973. Soils and Soil Fertility. McGraw-Hill, New York. 495 pp.

Walsh, G.E., D.E. Weber, L.K. Brashers, and T.L. Simon. 1990. Artificial sediments for use in tests with wetland plants. Environ. Exp. Bot. 30: 391-396.

Walsh, G.E., D.E. Weber, L.K. Esry, and M.J. Nguyen. 1991a. Synthetic sediments: a tool for research. pp. 5-40 to 5-47, *in*: Proceedings of the fifth Federal Interagency Sedimentation Conference, S.-S. Fan and Y.-H. Kuo, eds. Federal Energy Regulatory Commission, Washington, D.C.

Walsh, G.E., D.E. Weber, M.T. Nguyen, and L.K. Esry. Responses of wetland plants to effluents in water and sediment. Environ. Exp. Bot. *in press*.

Walsh, G.E., D.E. Weber, T.L. Simon and L.K. Brashers. 1991b. Toxicity tests of effluents with marsh plants in water and sediment. Environ. toxicol. Chem. 10: 517-525.

Walsh, G.E., D.E. Weber, T.L. Simon, L.K. Brashers, and J.C. Moore. Use of marsh plants for toxicity testing of water and sediment. In: Second Symposium on Use of Plants for Toxicity Testing of Water and Sediment, J. Gorsuch and W. Wang, eds. ASTM, Philadelphia. *in press*.

White, R.E. 1987. Introduction to the Principles and Practice of Soil Science, 2nd ed. Blackwell Sci. Pub., Oxford. 244 pp.

APPENDIX 1

TOXICITY TESTS OF EFFLUENTS WITH MARSH PLANTS IN WATER AND SEDIMENT

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TOXICITY TESTS OF EFFLUENTS WITH MARSH PLANTS IN WATER AND SEDIMENT

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Abstract—Methods are described for toxicity testing of water and sediment with two varieties of the freshwater marsh plant *Echinochloa crusgalli* (Linneaus) Palisot de Beauvois (Poaceae), and complex effluents. Two tests are described: a seed germination and early seedling growth test in water, and a survival and seedling growth test in natural and synthetic sediments. Effects of effluents from a sewage treatment plant, tannery, textile mill, pulp and paper mill, coking plant and sewage treatment plant included inhibition of germination, chlorophyll synthesis and growth. The tests with rooted marsh plants were sensitive to pollutants and detected toxicity of a range of pollutants in water and sediment. Synthetic sediments similar to natural sediments allowed toxicity tests to be done under carefully controlled conditions of particle size distribution, organic content, pH, electrode potential (Eh) and cation exchange capacity (CEC).

Keywords—*Echinochloa* Effluents Germination Survival Growth

INTRODUCTION

Freshwater and salt-marsh sediments may serve as sinks for industrial, municipal and agricultural pollutants. The pollutants, usually carried to marshes by rivers, tides and longshore currents, are partitioned between sediment particles and pore water [1]. In sediment, relative amounts of sand, silt, clay and organic matter (particulate and dissolved) and salinity of pore water affect partition coefficients of pollutants between pore water and sediment particles. Because distribution of the sediment components differs greatly among marshes and even within a single marsh, it is often difficult or impossible to predict effects of pollutants on marshes with accuracy. Also, as most marshes are contaminated to some extent, it is difficult to obtain sediment that permits study of possible effects of single substances or effluents without interference from other toxic substances.

This report describes methods for examination of effects of single toxicants, mixtures of toxicants and complex effluents in freshwater whole sediment and in pore water. It describes formulation of synthetic sediments that simulate natural sediments with regard to relative amounts and particle sizes of sand, silt, clay and particulate organic matter. It also describes a method for analysis of small amounts of pore water. The experimental methods utilize rooted marsh plants and the processes of seed germination and early seedling growth in water under conditions of light and darkness, and of seedling survival and growth in contaminated sediments. Although used here with vascular marsh plants, the artificial sediments may also be used in studies with other sediment-associated species.

MATERIALS AND METHODS

Test species

Two varieties of the common freshwater marsh grass *Echinochloa crusgalli* (Linneaus) Palisot de Beauvois (Poaceae) were used: var. *crusgalli* and var. *zelayensis*. Seeds were obtained from Wildlife Nurseries, Oshkosh, Wisconsin, and stored dry at 4°C. They were identified in our laboratory by growing to seed with confirmation of the varietal name according to the descriptions of Correll and Correll [2].

Effluents

Liquid effluents (Table 1) were collected in glass or polyethylene containers by personnel of EPA Region IV or of state agencies. The samples were

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Publication No. 685 from the Gulf Breeze Laboratory.
Use of trade names in this report is for identification purposes only and does not constitute endorsement by the U.S. Environmental Protection Agency.

Table 1. Chemical and physical characteristics of effluents used in plant tests

Effluent	Color	Odor	Suspended solids	pH	Salinity (‰)	Ammonia N (μg/L)	Nitrite N (μg/L)	Nitrate N (μg/L)	Phosphate P (μg/L)
Sewage	pink	none	yes	7.6	0	1,945	2.0	1.4	46.0
Textile	none	none	yes	8.2	0	25.0	2.4	20.5	21.2
Pulp and paper	brown	none	yes	7.8	0	10.6	50.9	10.5	35.4
Plating	none	slight	no	9.2	0	132	2.3	10.8	0.7
Coke	brown	strong	yes	7.9	0	157	0	0	5.7
Tannery	brown	none	yes	8.1	8	460	2.2	10.5	1.1

packed in ice, shipped to the Gulf Breeze laboratory in insulated containers and stored at 4°C immediately upon receipt. They were received within 24 h of shipment, and tests were begun the day after receipt. Visual examination was made for color and suspended particles, and presence or absence of odor was recorded; salinity was determined with an American Optical Co. temperature-compensated refractometer, and pH with a Beckman Co. Phi 12 pH/ISE meter. Nitrogen in ammonia, nitrite and nitrate, and phosphorus in phosphate, were determined by the methods of Parsons et al. [3].

Seed germination and early growth

Seed germination and early growth tests were performed in 47-mm clear polystyrene petri dishes with tightly capped lids (Millipore Corp., Bedford, MA). Seeds were surface-sterilized by immersion in 1% sodium hypochlorite for 20 min and rinsed in deionized water. Twelve seeds of *E. crusgalli* were placed in 10 ml of deionized water (freshwater control), undiluted effluent or effluent diluted with deionized water in the petri dishes. Each control and exposure concentration of effluent was prepared in triplicate. Thus, 36 seeds of *E. crusgalli crusgalli* and *E. crusgalli zelayensis* were used in controls and each treatment. In tests with tannery and pulp and paper mill effluents, seed germination and early growth tests were conducted only in light (approximately 35 $\mu E/m^2/s$ on a cycle of 8:16 h dark:light. Also, because tannery effluent was of 8‰ salinity, solutions of the same salinities as the effluent and its dilutions prepared with NaCl were tested. Duplicate tests were conducted with textile mill, sewage plant, coking plant and metals plating plant effluents under the same light regime and in total darkness. Germination tests were conducted for 7 d, and germinated seedlings were counted at selected intervals between day 2 of exposure and the end of the test. At the end of the exposure period, the remains of the bracts were removed from each seedling. The seedlings from each petri dish were combined and dried for 24 h at 103°C and weighed to the nearest 0.1 mg on a Mettler Model AE 163 balance. Weights of exposed seedlings were compared to weights of control seedlings.

Survival and growth

Sediments. Natural freshwater sediment was collected from a wetland near Milton, Florida. Leaves, twigs and other large particles were removed, and the sediment was dried in air. Particle size distribution of the natural sediment was determined by sieving and by settling rate in water [4]. Organic content was determined by ashing at 550°C for 24 h.

Synthetic sediments were formulated from washed sand, silt, clay and organic matter. Sand (Tables 2 and 3) was obtained from New England Silica, Inc., South Windsor, Connecticut. Three types of sand were used: Mystic White® No. 85 (fine), No. 45 (medium) and No. 18 (coarse). Each type was sieved to obtain the proper grain size for use in synthetic sediments. Silts and clays (Tables 2 and 3), manufactured by Englehard Corp., Edison, New Jersey, were obtained from Gulf Coast

Table 2. Composition[a] of sand, silt and clay used in formulation of artificial sediments

Sand (as oxides)	%	Clay and silt	%
SiO_2	97.70	SiO_2	65.9
Al_2O_3	1.50	Al_2O_3	12.2
K_2O	0.29	MgO_2	11.5
CaO_2	0.08	$CaCO_3$	4.3
Fe_2O_3	0.07	Fe_2O_3	3.6
MgO_2	0.06	P_2O_5	1.1
TiO	0.04	K_2O	0.8
Na_2O	0.01	TiO	0.5
Loss on ignition	0.25	Trace elements	0.1

[a]Expressed as percentage by weight.

Table 3. Particle size distribution of natural[a] and synthetic sediments used in toxicity tests with rooted freshwater plants

Class	Particle size (μm)	Weight %	
		Natural	Synthetic
Coarse sand	500–1,500	0.6	0.6
Medium sand	250–499	9.5	8.7
Fine sand	63–249	67.4	69.2
Silt	4–62	10.3	10.2
Clay	<4	6.7	6.4
Organic matter	–	4.9	4.9

[a]0.6% of weight of natural sediments was lost during handling.

Chemical Corp., Tampa, Florida. Particulate organic matter was composed of commercial peat humus milled to pass the 840 μm (20-mesh) screen on a Wiley mill.

After particle size analysis of the natural sediment, the sand, silts, clays and organic matter were used to formulate a sediment with similar particle size ratios and organic contents (Table 3). Natural and synthetic sediments were hydrated by mixing sediment into deionized water or effluent at the ratio of 42 ml of water or effluent per 135 g of sediment. Survival and growth of seedlings in sediments hydrated with saline solution, as described above, were also determined when tannery effluent was tested. Mixing was by spatula in a glass beaker, and the mixture was stirred until smooth and homogeneous.

After hydration, approximately 100 ml of sediment was apportioned to each of three Styrofoam® cups, 5.5 cm high × 7.4 cm diameter. This yielded a system in which the sediment was overlain by approximately 5 mm of water or effluent. In tests with effluents from a textile mill, plating works and coking plant, three cups received an additional 20 ml of effluent at the surface of the sediment at 5-d intervals between planting of seedlings and harvest. Three other cups received 20 ml of deionized water. Sediment pH was measured [5] immediately before planting the seedlings. Redox potential (Eh) was measured with a Radiometer/Copenhagen PHM pH meter fitted with a platinum electrode [5], and cation exchange capacity (CEC) was measured by the ion exchange procedure [6] in natural and synthetic sediments prepared with deionized water. The Eh and CEC of natural and synthetic sediments were similar (Table 4). The pH of natural sediment (5.8) was lower than that of synthetic sediment (7.5) (Table 4), but, as discussed below, there were no statistically significant differences in responses to effluents in natural or synthetic sediments.

Table 4. Redox potential (Eh), cation exchange capacity (CEC) and pH of natural and synthetic sediments used in toxicity tests with rooted freshwater plants

	Natural	Synthetic
Eh, mV	380	315
CEC, meq/100 g	16.6	19.0
pH	5.8	7.5

Exposure of seedlings. Seeds were soaked in 1% sodium hypochlorite for 20 min, rinsed with deionized water and set to germinate 4 d before tests were started. *E. crusgalli* was germinated in deionized water as in the seed germination and early growth tests.

Twelve seedlings were planted in each cup, and each control and treatment was done in triplicate. Seedlings were placed into holes in the sediment with their coleoptiles above the surface of the sediment. Roots remained intact during planting. Twenty milliliters of Hoagland nutrient solution [7] was added to the surface of each sediment.

Echinochloa crusgalli was grown for two weeks under the temperature and lighting conditions given above. At the end of the growth period, surviving seedlings were enumerated and collected carefully by peeling the Styrofoam cup from the sediment and washing the sediment from the roots. Remains of bracts were removed, and the seedlings of each cup were combined, dried and weighed as described above.

Statistical analyses

Data were evaluated statistically by a general linear model for analysis of variance (ANOVA). When F values of the ANOVA were significant ($P = 0.05$), means of control and treated groups were compared by Tukey's Studentized Range Test [8], which allowed for calculation of the lowest observed effect concentration (LOEC) ($\alpha = 0.05$). In the test with tannery effluent, effects of the effluent and saline water on growth were compared by analysis of covariance [8] to separate the effects of salinity from those of the effluent. This was possible due to the linear growth response to salt concentrations.

RESULTS

Sewage treatment plant

Germination and early growth. Undiluted sewage treatment plant waste inhibited germination and early growth of *E. crusgalli* var. *crusgalli* and var. *zelayensis* (Table 5). There was total inhibition in light through 4 d of exposure, but by day 6, germination percentages were similar in control and treated groups. Average weights of treated seedlings were significantly lower than those of controls because they germinated later. Germination was inhibited in darkness throughout the test, suggesting that the toxicant(s) was photolabile.

Survival and growth. One application of sewage treatment plant effluent to natural and synthetic sediments had no effect on survival or growth of either variety.

Textile mill

Seed germination and early growth. Textile mill effluent had no effect on germination of *E. crusgalli* var. *crusgalli* or var. *zelayensis*. Percentage germination was not significantly different in controls and effluent in light and darkness between 2 and 7 d of exposure.

The effluent inhibited seedling growth by both varieties in the light, but not in darkness (Fig. 1). The LOEC for var. *crusgalli* was 50% effluent; for var. *zelayensis* it was 75%.

Survival and growth. Textile mill effluent had no effect on survival and growth of either variety in natural or synthetic sediment.

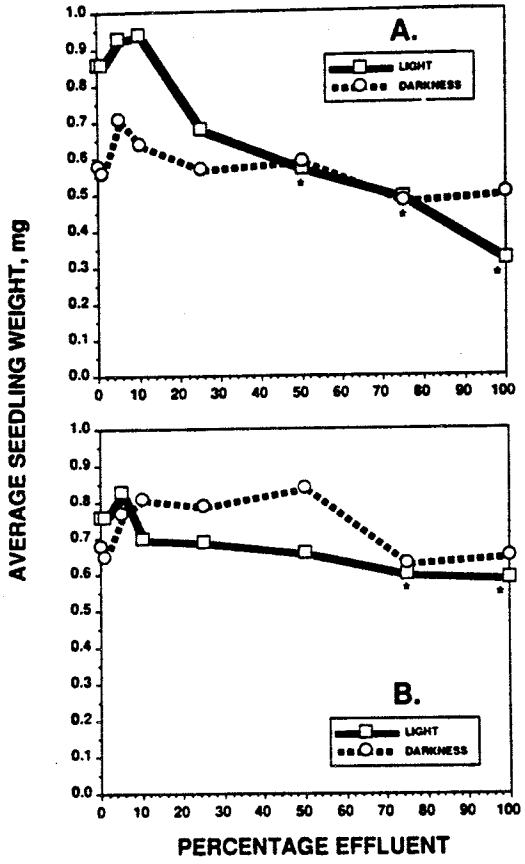

Fig. 1. Average weights of seedlings of *Echinochloa crusgalli* var. *crusgalli* (**A**) and var. *zelayensis* (**B**) exposed for 7 d to dilutions of textile mill effluent in early growth tests. ★ = significantly different from control in light; $P = 0.05$.

Table 5. Effects of undiluted sewage treatment plant effluent on germination and seedling weight of *Echinochloa crusgalli* var. *crusgalli* and var. *zelayensis* in light and darkness

Variety	Treatment	Percent seed germination (days of exposure) 2 d	4 d	6 d	7 d	Seedling wt.[a] (mg)
crusgalli	control, light	86.1	86.1	86.1	86.1	0.8
	effluent, light	0[b]	0[b]	75.0	83.3	0.2[b]
	control, darkness	80.6	94.4	94.4	94.4	0.5
	effluent, darkness	0[b]	0[b]	0[b]	8.3[b]	<0.1[b]
zelayensis	control, light	94.4	94.4	94.4	94.4	0.9
	effluent, light	0[b]	0[b]	86.1	91.7	0.3[b]
	control, darkness	83.3	94.4	97.2	97.2	0.6
	effluent, darkness	0[b]	0[b]	8.3[b]	11.1[b]	<0.1[b]

Thirty-six seedlings were used in each test.
[a]At Day 7.
[b]Statistically different from control ($P = 0.05$) of same treatment.

Pulp and paper mill

Seed germination and early growth. Effluent from a pulp and paper mill had no effect on germination of *E. crusgalli* var. *crusgalli* and var. *zelayensis* in light. The test was not done in darkness. The effluent did inhibit early growth of seedlings (Fig. 2), and the LOECs were 75% effluent for both varieties.

Survival and growth. One treatment of sediments with undiluted effluent did not affect survival and growth of either species.

Metals plating works

Germination and early growth. Metals plating works effluent did not affect the germination rate of *E. crusgalli* var. crusgalli, but it did inhibit germination of var. *zelayensis* (Table 6). The LOEC increased between 2 and 7 d of exposure. In light, the LOEC on days 2 and 3 was 50% effluent, on days 4 and 5 it was 100% and there was no effect on days 6 and 7. Toxicity to germination was slightly greater in darkness: The LOEC rose from 25% on day 2 to 75% on day 7.

The effluent also caused significant reduction in weight of seedlings of both varieties in light and darkness (Fig. 3). The LOEC for each was 50% effluent, except for var. *zelayensis* in darkness, during which it was 75%..

Survival and growth. Metals plating works effluent had no effect on survival of seedlings in natural and synthetic sediments. Seedling weight was reduced significantly by the first and third treatments with undiluted effluent (Table 7).

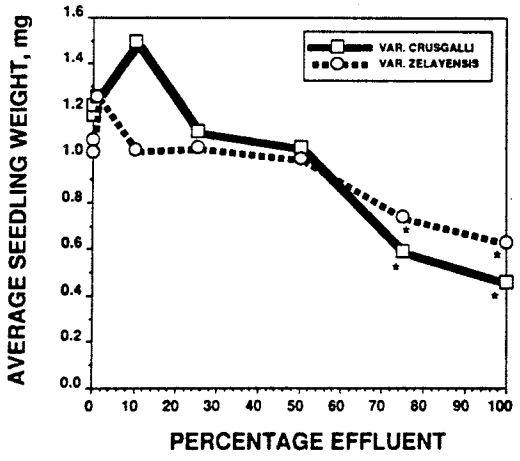

Fig. 2. Average weights of seedlings of *Echinochloa crusgalli* var. *crusgalli* and var. *zelayensis* exposed to pulp and paper mill effluent in light in early growth tests. ★ = significantly different from control, $P = 0.05$.

Table 6. Percentage of *Echinochloa crusgalli* var. *zelayensis* seedlings germinated in metals plating works effluent in light and darkness

	Percentage germination (days of exposure)					
Dilution	2 d	3 d	4 d	5 d	6 d	7 d
In light						
Control	44.4	80.6	86.1	86.1	86.1	86.1
1	66.7	91.7	97.2	97.2	97.2	97.2
5	69.4	80.6	86.1	86.1	86.1	88.9
10	61.1	83.3	86.1	88.9	91.7	94.4
25	38.9	69.4	75.0	77.8	77.8	77.8
50	13.9[a]	50.0[a]	69.4	91.7	91.7	91.7
75	0[a]	33.3[a]	63.9	83.3	88.9	94.4
100	0[a]	19.4[a]	25.0[a]	55.6[a]	80.6	83.3
In darkness						
Control	16.7	41.7	52.8	52.8	55.6	55.6
1	47.2	63.9	69.4	75.0	75.0	75.0
5	22.2	41.7	52.8	55.6	61.1	61.1
10	38.9	50.0	61.1	66.7	66.7	66.7
25	11.1[a]	19.4[a]	25.0[a]	41.7	44.4	44.4
50	0[a]	25.0[a]	25.0[a]	27.8[a]	30.6	30.6
75	0[a]	8.3[a]	11.1[a]	16.6[a]	19.4[a]	22.2[a]
100	0[a]	0.1[a]	13.9[a]	16.6[a]	22.2[a]	25.0[a]

Thirty-six seeds were exposed in each control and treatment.
[a]Significantly less than control, $P = 0.05$.

Coking plant

Germination and early growth. Coking plant effluent inhibited germination of both varieties of *E. crusgalli*, and its effect was greater in var. *zelayensis* (Table 8). The effluent LOECs after 2 d of exposure for var. *crusgalli* were 75% effluent (light) and 50% (darkness). For var. *zelayensis* they were 5% (light) and 10% (darkness). The LOECs became greater with time of exposure; by day 7 they were 100% effluent in light and darkness for var. *crusgalli* and 10% (light) and 50% (darkness) for var. *zelayensis*.

Early growth of seedings in light and darkness also was inhibited by coking plant effluent, and growth of both varieties was inhibited more strongly in light than it was in darkness (Fig. 4). The LOEC concentrations were considerably lower for weight than they were for germination after 7 d of exposure, at which time they were 5% effluent (light) and 5% (darkness) for var. *crusgalli* and 1% (light) and 5% (darkness) for var. *zelayensis*. Growth of var. *zelayensis* was inhibited completely by 75% effluent.

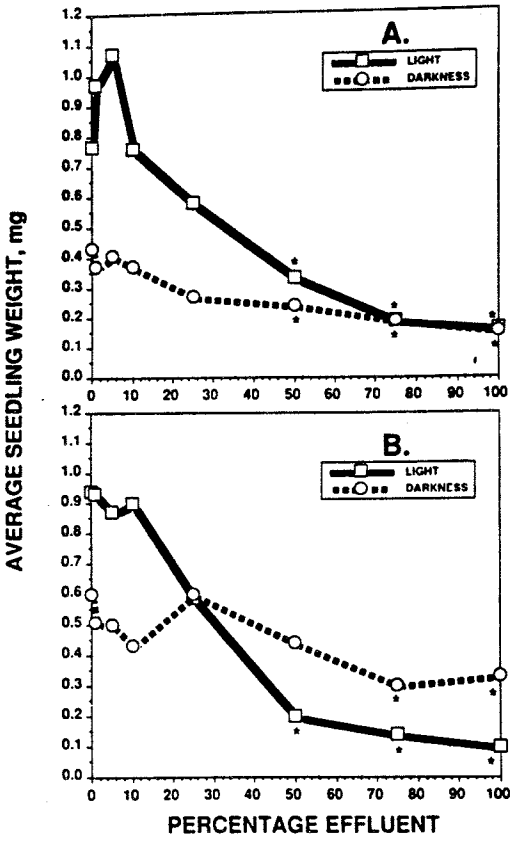

Fig. 3. Average weights of seedlings of *Echinochloa crus-galli* var. *crusgalli* (**A**) and var. *zelayensis* (**B**) exposed for 7 d to dilutions of plating works effluent in early growth tests. ★ = significantly different from control, $P = 0.05$.

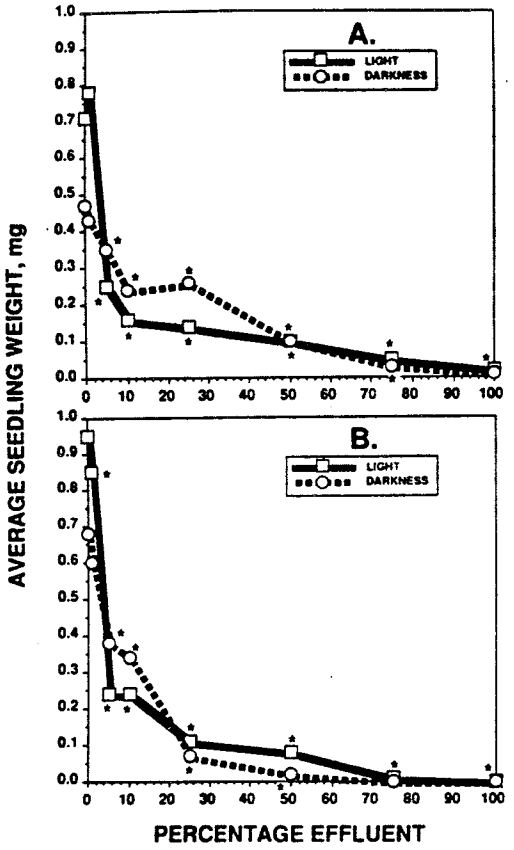

Fig. 4. Average weights of seedlings of *Echinochloa crus-galli* var. *crusgalli* (**A**) and var. *zelayensis* (**B**) exposed for 7 d to dilutions of coking plant effluent in early growth tests. ★ = significantly different from control, $P = 0.05$.

Effects of the effluent were greater in light than they were in darkness, suggesting the presence of a nonlabile inhibitor of photosynthesis. It is likely that inhibition of photosynthesis was caused by inhibition of chlorophyll synthesis. Plants in all of the exposure concentrations were white and without any visual trace of green pigmentation.

Survival and growth. The first and third treatments of natural and synthetic sediments, respectively, with undiluted effluent had no effect on survival of either variety. They did, however, inhibit growth significantly (Table 7).

Tannery

Germination and early growth. There were no effects of undiluted tannery effluent and effluent diluted with 8‰ salinity water or water of 8‰ salinity on germination. There was an inverse relationship between concentration of effluent or salt and average seedling weight (Fig. 5). The LOECs were 75% effluent (equal to 6‰ salinity), diluted with deionized water, and 4‰ salinity water. Because 8‰ salinity was toxic, all seedlings exposed to effluent diluted with 8‰ salinity water were significantly lower in weight than those in the deionized water control.

Survival and growth. Tannery effluent and saline water did not inhibit survival in synthetic sediment, but growth of var. *crusgalli* was inhibited (Table 7, Fig. 6). Variety *zelayensis* was not tested. When effluent was diluted with water of 8‰ salinity, seedling weights were depressed strongly at all waste concentrations (Fig. 6). The LOEC for effluent diluted with deionized water was 50% (equal to 4‰ salinity); the LOEC for saline water was 4‰. Those data indicate that salt was the major toxic factor for inhibition of growth by tannery effluent. However, analysis of covariance of effluent and

Table 7. Average weights and percentage inhibition of growth of *Echinochloa crusgalli* var. *crusgalli* and var. *zelayensis* exposed to one and three treatments with industrial effluents in natural and synthetic sediments

Effluent	Variety	Sediment	Treatments	Avg. wt. (mg)	Inhibition (%)
Plating	*crusgalli*	natural	0 (control)	9.0	–
			1	5.9[a]	34.4[a]
			3	6.0[a]	33.3[a]
		synthetic	0 (control)	16.1	–
			1	11.3[a]	29.8[a]
			3	6.1[a]	62.1[a]
	zelayensis	natural	0 (control)	8.6	–
			1	5.6[a]	34.9[a]
			3	7.7	11.1
		synthetic	0 (control)	9.9	–
			1	7.0[a]	29.3[a]
			3	7.0[a]	29.3[a]
Coke	*crusgalli*	natural	0 (control)	10.2	–
			1	3.8[a]	62.7[a]
			3	3.6[a]	64.7[a]
		synthetic	0 (control)	15.8	–
			1	7.7[a]	51.3[a]
			3	6.0[a]	62.0[a]
	zelayensis	natural	0 (control)	9.1	–
			1	4.4[a]	51.5[a]
			3	4.2[a]	53.8[a]
		synthetic	0 (control)	12.3	–
			1	10.0[a]	18.7[a]
			3	7.8[a]	36.6[a]
Tannery	*crusgalli*	synthetic	0 (control)	19.0	–
			1	3.0[a]	84.2[a]

[a]Statistically significant inhibition compared to control ($P = 0.05$) of same sediment.

salt concentration demonstrated that depression of average seedling weight was greater in diluted effluent than it was in saline water of comparable salinity at effluent concentrations of 50% (4‰ salinity) and above. This indicates that another, unidentified toxic factor was present in the effluent. The factor was not pH, which did not affect response to effluent or salinity (Fig. 6).

DISCUSSION

Rooted marsh plants are shown here to be useful experimental organisms for detection of toxicity of effluents and herbicides in water and sediments. They are responsive in a variety of ways and can be tested in natural sediments and artificial sediments of any desired formulation. Toxic end points, such as germination, rate of early growth in light and darkness, survival and later growth, are easily measured [9]. Germination and early growth studies can be conducted in a small volume of water and can thus be used to test toxicity of sediment pore water. Germination and growth are separate physiological events; tests in light and darkness allow identification of effects on imbibition of water and cell elongation (germination) and mobilization of nutrients and photosynthesis (growth). In addition, photolabile toxicants can be identified by such tests.

The tests described below demonstrate responses of marsh plants to toxicants in water and sediments:

Sewage treatment plant effluent inhibited germination of seeds, but the toxic factor was photolabile and the effect was lost after 4 d of exposure in light.

Textile and pulp and paper mill effluents had no effect on germination. They inhibited early seedling growth in light but not in darkness.

Metals plating works effluent inhibited germination of one plant variety and early growth of both varieties in light and darkness. It also reduced growth rates of both varieties in natural and synthetic sediments.

Coking plant effluent inhibited both germination and early growth, with greater effects in light

Table 8. Number of *Echinochloa crusgalli* var. *crusgalli* and var. *zelayensis* seeds germinated in coking plant effluent in light and darkness

% Effluent	No. germinated in light (days of exposure)						No. germinated in darkness (days of exposure)					
	2	3	4	5	6	7	2	3	4	5	6	7
crusgalli												
Control	32	32	32	32	32	32	36	36	36	36	36	36
1	35	35	35	35	35	35	32	33	33	33	33	33
5	29	29	29	29	29	32	32	33	33	33	33	33
10	29	31	31	31	32	32	29	30	31	32	32	32
25	24	30	30	30	31	31	25	26	27	27	27	27
50	30	32	32	32	32	33	14[a]	18[a]	23	23	24	25
75	11[a]	26	27	28	28	28	4[a]	10[a]	15[a]	20	20	20
100	3[a]	10[a]	15[a]	19[a]	19[a]	19[a]	0[a]	2[a]	7[a]	12[a]	12[a]	13[a]
zelayensis												
Control	33	36	36	36	36	36	9	11	12	17	17	17
1	30	32	34	35	35	35	10	13	15	15	15	15
5	14[a]	25	31	33	33	33	6	14	19	19	20	21
10	9[a]	18[a]	21[a]	25[a]	25[a]	25[a]	0[a]	3[a]	3[a]	7[a]	8	9
25	5[a]	10[a]	18[a]	31	34	35	0[a]	2[a]	2[a]	3[a]	5[a]	8
50	0[a]	2[a]	7[a]	18[a]	18[a]	22[a]	0[a]	0[a]	1[a]	1[a]	1[a]	3[a]
75	0[a]	1[a]	2[a]	7[a]	7[a]	8[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]
100	0[a]	0[a]	0[a]	2[a]	2[a]	2[a]	0[a]	0[a]	0[a]	0[a]	0[a]	0[a]

Thirty-six seeds were used in each control and effluent concentration.
[a]Significantly less than control, $P = 0.05$.

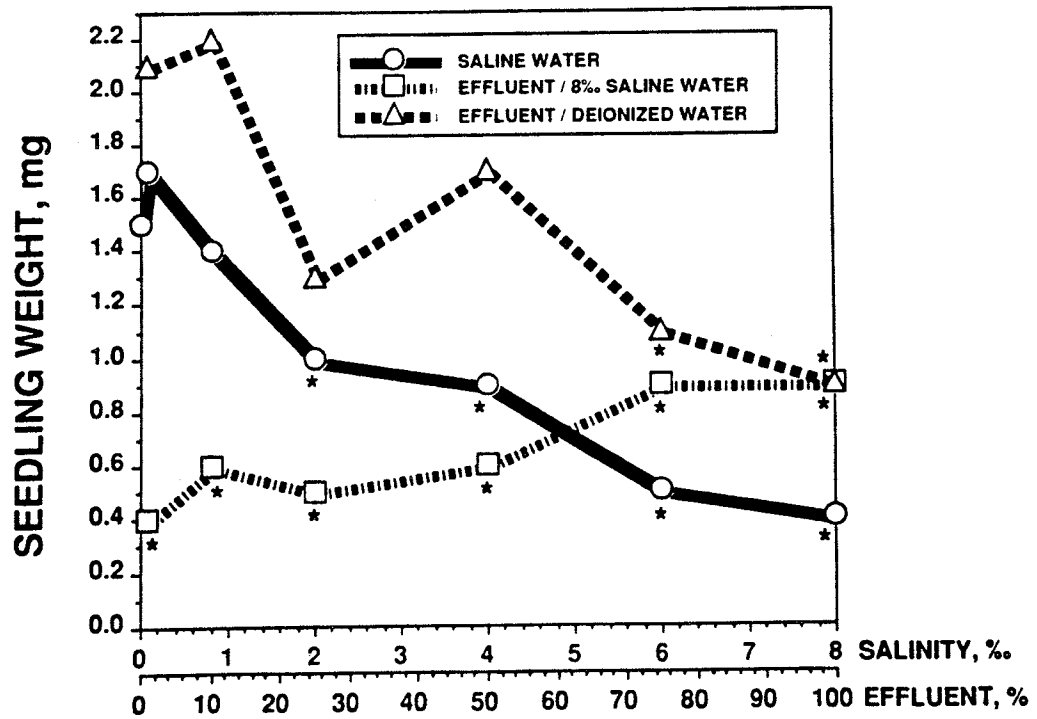

Fig. 5. Average weights of *Echinochloa crusgalli* var. *crusgalli* seedlings grown in saline water, tannery effluent diluted with 8‰ saline water and tannery effluent diluted with deionized water. ★ = significantly different from control, $P = 0.05$.

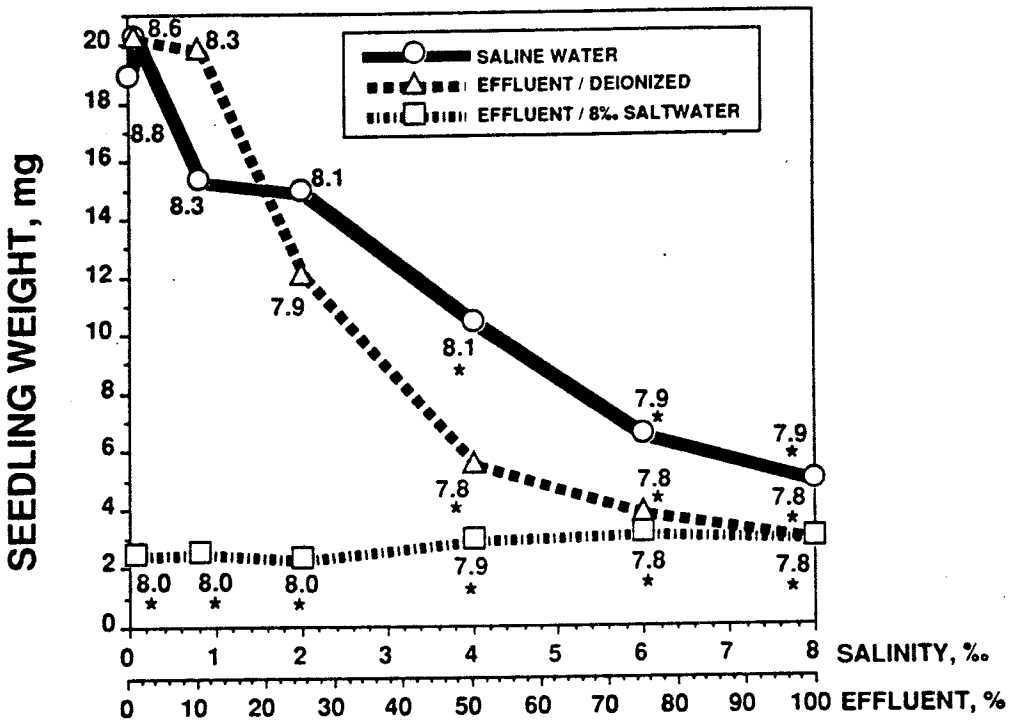

Fig. 6. Average weights of *Echinochloa crusgalli* var. *crusgalli* seedlings exposed to saline water, tannery effluent diluted with 8‰ saline water and tannery effluent diluted with deionized water in synthetic sediment. Number at each point = pH of sediment. ★ = significantly different from control, $P = 0.05$.

because it inhibited chlorophyll synthesis. It also inhibited growth in natural and synthetic sediments.

Tannery effluent inhibited early and later seedling growth.

CONCLUSIONS

Germination, survival and growth of freshwater marsh plants were inhibited by effluents in standardized tests with water and sediment. The tests described here provide reliable toxicity data for estimation of potential effects of effluents in marshes and can be used for regulation of effluent discharges to natural systems.

Acknowledgement—We thank Mr. William Peltier of EPA Region IV, Athens, Georgia, for providing the effluents used in the tests.

REFERENCES

1. **Carsel, R.F.** 1989. Hydrologic processes affecting the movement of organic chemicals in soils. In B.L. Sawhney and K. Brown, eds., *Reactions and Movement of Organic Chemicals in Soils*. SSSA Special Publication No. 22. Soil Science Society of America and American Society of Agronomy, Madison, WI, pp. 439–445.
2. **Correll, D.S.** and **H.B. Correll.** 1972. Aquatic and wetland plants of southwestern United States. Water Pollution Control Research Series 16030 DNL 01/72. U.S. Environmental Protection Agency, Washington, DC.
3. **Parsons, J.R., Y. Morita** and **C.M. Lalli.** 1984. *A Manual of Chemical and Biological Methods for Seawater Analysis.* Pergamon Press, Elmsford, NY.
4. **Folk, R.L.** 1980. *Petrology of Sedimentary Rocks.* Hemphill Publishing, Austin, TX.
5. **Jackson, M.L.** 1960. *Soil Chemical Analysis.* Prentice-Hall, Englewood Cliffs, NJ.
6. **U.S. Department of Agriculture.** 1972. Soil survey laboratory methods and procedures for collecting soil samples. Soil Survey Investigations Report No. 1. Soil Conservation Service, Washington, DC.
7. **Hoagland, D.R.** and **D.I. Arnon.** 1950. The Water Culture Method for Growing Plants without Soil. California Agricultural Experiment Station Circular 347. Berkeley, CA.
8. **SAS Institute.** 1982. *SAS® User's Guide: Statistics, 1982 Edition.* Cary, NC.
9. **Walsh, G.E., D.E. Weber, T.L. Simon, L.K. Brashers** and **J.C. Moore.** 1991. Use of marsh plants for toxicity testing of water and sediment. *Second Symposium on Use of Plants for Toxicity Assessment.* American Society for Testing and Materials, Philadelphia, PA (in press).

APPENDIX 2

USE OF MARSH PLANTS FOR TOXICITY TESTING OF WATER AND SEDIMENT

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Use of Marsh Plants for Toxicity Testing of Water and Sediment[2]

ABSTRACT: The freshwater wetland plants, *Echinochloa crusgalli crusgalli* and *Echinochloa crusgalli zelayensis*, and the saltmarsh plant, *Spartina alterniflora*, were exposed to the herbicides, metolachlor and norflurazon, in two types of toxicity tests: seed germination and early seedling growth in water, and seedling survival and growth in natural and artificial sediments. The artificial sediments were formulated to simulate the natural sediments with regard to particle size distribution and organic content. The herbicides did not affect rate of germination, but significantly inhibited rate of early growth, and survival and rate of growth of older seedling in sediments. *Echinochloa* was more sensitive than *Spartina* to both herbicides. Inhibition of the growth rates of the two varieties of *E. crusgalli* was similar in natural and simulated sediments, but inhibition of growth of *S. alterniflora* was greater in simulated than in natural sediment. It is concluded that the

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species tested may be used for estimation of potential effects of toxicants on wetland plants and that simulated sediments of known composition may be used in sediment toxicity tests.

KEYWORDS: wetland plants, Echinochloa crusgalli, Spartina alterniflora, metolachlor, norflurazon, germination, survival, growth, natural sediment, simulated sediment

Freshwater and estuarine wetlands serve as sinks for waterborne pollutants [1]. These pollutants may be in the dissolved state, adsorbed to suspended particles [2], or bound to dissolved organic matter [3], but the ultimate locations of many pollutants are in interstitial water or on particles of wetland sediments [4]. Whether dissolved or adsorbed to particulates, toxic substances in sediments can be taken up by the roots of wetland plants and translocated to aerial organs, where they may inhibit growth, injure foliage or kill the plants [5]. Also, wetland plants generally produce numerous seeds which germinate at the sediment-water interface, where they may be exposed to toxic substances in water.

Rooted plants are the dominant life forms that control the physical, chemical, and biological characteristics of wetland ecosystems. They are the major primary producers and sources of detritus, their roots and rhizomes stabilize sediment, and they provide food and habitat for animals. Chemical hazard to rooted wetland plants also constitutes a threat to their ecosystems. Such hazards could occur through effects of toxic substances on seed germination, seedling growth, and survival.

Few studies describe effects of toxic substances in sediments on wetland plants [6], and we are not familiar with methods devoted specifically to development of artificial sediments for toxicity testing with such plants. The research reported here was designed to (1) develop methods for exposure of freshwater and estuarine marsh plants to toxicants in water and sediment, (2) identify marsh plant species that can be used in toxicity tests, and (3) conduct toxicity tests in natural and artificial sediments with substances known to be toxic to plants.

Use of artificial sediments was deemed critical to evaluation of effects of toxicants in sediments. In early studies, we found natural sediments to be unacceptable for toxicity tests because while wet and without amendation, pH decreased with time and weed seeds germinated. When dried in air, pHs of reconstituted natural sediments were as low as 2, and weed seeds continued to germinate. Moreover, structure of natural sediments could not be varied experimentally, they contained unknown quantities of nutrients and, perhaps, toxicants. We required sediments whose properties could be controlled to simulate the variety of sediments found in nature. Artificial sediments that we formulated varied in grain size distribution and organic content. Their characteristics and formulation methods are reported here.

The methods for toxicity testing of plants and sediments consisted of (1) a germination and early growth test in water, and (2) a seedling survival and growth test in natural and artificial sediments. Although the natural sediments were altered by drying, they were used as a standard to which tests with artificial sediments were compared. Two varieties of a freshwater marsh species and one species of estuarine plant were tested with two herbicides.

Effects of the herbicides in water, natural, and artificial sediments are reported and the value of the tests is discussed.

Materials and Methods

Plant Species

Freshwater - The common freshwater wetland plant, *Echinochloa crusgalli* (Linneaus) Palisot de Beavois, (Gramineae) was used. Two varieties, *crusgalli* and *zelayensis*, were obtained as seed from Wildlife Nurseries, Oshkosh, Wis., and stored dry at approximately 4°C. The varietal names were confirmed by growing plants to seed, with identification according to Correll and Correll [7].

Estuarine - *Spartina alterniflora* Loisel (Gramineae) seeds were obtained from Environmental Concern, St. Michaels, Md. Upon receipt, the dry seeds were placed in natural seawater diluted with deionized water to 4 parts per thousand (ppt) salinity and stored at 4°C. Identity was confirmed from the description given by Hotchkiss [8].

Toxicity Tests

Germination and Early Growth - Seed germination and early growth tests were performed in 47-mm clear polystyrene Petri dishes with tightly capped lids (Millipore Corp., Bedford, MA). Seeds were surface-sterilized by immersion in 1% sodium hypochlorite for 20 min and rinsed in deionized water. Twelve seeds of *Echinochloa* were placed in 10 ml of deionized water (freshwater control) and up to 7 concentrations of herbicide in Petri dishes. Each control and

exposure concentration was prepared in triplicate. Thus, 36 seeds were exposed in the control and each treatment. Tests with *Echinochloa* were conducted for 7 days under cool white fluorescent lights at approximately 35 $\mu E/m^2/sec$ with a diel cycle of 16 h light: 8 h darkness. With *Spartina*, eight surface-sterilized seeds were placed in 10 ml of 4 ppt diluted seawater (seawater control) and up to 7 dilutions of herbicide. Controls and exposure concentrations were prepared in triplicate, so that 24 seeds of *Spartina* were used in each. The tests were conducted for 10 d under cool white fluorescent lights at approximately 35 $\mu E/m^2/sec$ and a temperature regime of 16 h at 18 ± 1°C and 8 h at 35 ± 1°C [9]. Germinated seedlings were enumerated each day. At the end of the exposure, the roots and stems were cut from the caryopses and the plant material from each Petri dish was combined and dried for 24 h at 103°C and weighed to the nearest 0.1 mg on a Mettler Model AE 103 balance.

Seedling Survival and Growth - Survival and growth tests were conducted in natural and artificial sediments. Natural freshwater sediment was collected from a marsh near Milton, Fla., and saltmarsh sediment was collected from a marsh near Pensacola, Fla. Leaves, twigs, and other large particles were removed and the sediments dried in air at room temperature. Particle size distributions were determined by dry sieving and by settling rate in water [10]. Organic content was determined by ashing at 550°C for 24 h. Artificial sediments were formulated to simulate the physical properties of the natural sediments (Table 1).

Simulated sediments were formulated from washed quartz sand, silt, clay, and organic matter. Fine, medium, and coarse sands (New England Silica, Inc., South Windsor, Conn.) were sieved to obtain the proper grain size for each

simulated sediment. Silts (average particle sizes 4.8 and 1.8 μm) and clays (average particle sizes 0.1 and 2.0 μm) were produced by Englehard Corp., Edison, N.J. Particulate organic matter was air-dried commercial peat humus (Greenleaf Products, Inc., Haines, Fla.) milled to an average particle size of 840 μm on a Wiley Mill.

The dry components of simulated sediments, mixed in the desired proportions, and air dried natural sediments were reconstituted for survival and growth studies by mixing with either deionized water or 4 ppt diluted seawater at the ratio of 42 ml water: 135 g sediment. Treated sediments were prepared with water that contained dissolved herbicide. Sediments were mixed with a spatula in a glass beaker until smooth and homogeneous. Approximately 100 ml of wet sediment were added to each of three styrofoam cups, 5.5 cm high x 7.5 cm diam. Sediment pH was determined by addition of 100 g sediment to 100 ml deionized water in a glass beaker. The mixture was stirred for one min and allowed to settle for one h, at which time pH was determined with a Beckman Phi 12 pH/ISE meter. Cation exchange capacity (CEC) was determined by the ion-exchange analysis procedure [12].

Young seedlings were used in the survival and growth tests. Seeds were surface-sterilized in 1% sodium hypochlorite and set to germinate 4 d (*Echinochloa*) or 10 d (*Spartina*) before tests were to begin. *Echinochloa* seeds were germinated in deionized water at 24 ± 1°C under cool white fluorescent lights at approximately 35 $\mu E/m^2/sec$ and a 16 h light: 8 h darkness cycle. *Spartina* was germinated in 4 ppt diluted seawater in a temperature regime of 16 h at 18 ± 1°C and 8 h at 35 ± 1°C with 16 h light and 8 darkness.

Twelve seedlings were planted in each cup and triplicate cups were prepared for each control and herbicide concentration was done in triplicate. Seedlings were planted in holes in sediment without damage to roots and with coleoptiles above the sediment. *Echinochloa* was grown for two weeks and *Spartina* for four weeks at 24 ± 1°C under cool white fluorescent lights at approximately 35 $\mu E/m^2/sec$ on a 16 h light: 8 h darkness cycle. Twenty ml of Hoagland solution [11] were added to *Echinochloa* immediately after planting and on the 4th and 12th d, and to *Spartina* immediately after planting and on the 4th, 12th, and 20th d. At the end of the growth period, surviving seeds were enumerated and collected carefully by peeling the styrofoam cup from the sediment and washing sediment from the roots with deionized water. Shoots and roots were cut from the caryopses and the plant material of each cup was dried at 103°C for 24 h and weighed to the nearest 0.1 mg.

Preparation of Herbicide Solutions

The herbicides, metolachlor (2-chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl) acetamide, 98% pure, from Ciba-Geigy Corp., Greensboro, N.C.) and norflurazon (4-chloro-5-methylamino-2-(3-trifluoromethylphenyl)-pyridazin-3 (2H) one, > 98% pure, Sandoz, Inc., Homestead, Fla.) were used without carrier. In seed germination and early growth tests, the highest concentration to be used was dissolved in deionized water or 4 ppt diluted seawater and diluted as needed. In seedling survival and growth tests, saturated solutions were prepared and diluted as needed for the desired concentration in sediment.

Purity of the herbicides and all concentrations in water were confirmed by gas chromatography. Concentrations in sediments were not confirmed because percentage recovery was very low. Samples of freshwater or diluted seawater containing herbicides were extracted with solvent or mixtures of solvents and analyzed by gas chromatographs equipped with packed columns and either electron-capture or nitrogen phosphorus detectors. Average recovery of these compounds spiked into freshwater and seawater to validate analyses of test water was greater then 85% for all compounds. Depending on concentrations and sensitivity of the detector, sizes of samples extracted with solvent ranged from 2 ml to 20 ml. The types and amounts of solvents were different for each compound and were as follows: metolachlor (hexane, 2-10 ml); norflurazon (40% ethyl acetate/60% hexane [v/v] 2-20 ml).

Statistical Analyses

Two statistical procedures were used for analysis of germination, survival, and weight data. The mean number of germinated seeds or surviving seedlings, and the average weights of seedlings per dish or cup were calculated. Comparisons of the means in each test, the lowest observed effect concentration (LOEC) and differences between each treatment within each test were computed with Tukey's Studentized Range Test [13]. Comparisons of responses in natural vs simulated sediments were made by two-way analysis of variance (ANOVA) [13], $\alpha = 0.05$.

Results and Discussion

ph and Cation Exchange Capacity

The ph of dried natural sediments was low (Table 2). Simulated sediments were slightly basic and had no added sulfur. It is probable that oxidation of sulfide in the wetland sediments contributed to the low pHs. Herbicidal activities at the pHs of the natural sediments are discussed below.

Cation exchange capacities of natural and simulated sediments were similar (Table 2).

Metolachlor

Seed Germination and Early Growth - Metolachlor did not affect the germination rate of either species. It did, however, suppress growth of both varieties of *E. crusgalli* and of *S. alterniflora* (Fig. 1, Table 3). The LOEC for metolachlor and seedling weights with *E. crusgalli crusgalli* and *E. crusgalli zelayensis* was 0.25 mg/L; for *S. alterniflora*, it was 0.5 mg/L. In each case, concentrations at and greater than the LOEC were not statistically significantly different from each other. The highest concentration in tests with *Echinochloa* was 20 times greater than the LOEC; with *Spartina*, the highest concentration was four times greater then the LOEC. This phenomenon, in which increasing concentrations of herbicide did not reduce final seedling weight, occurred in all tests with metolachlor and norflurazon. Metolachlor is a chloroacetamide preemergence herbicide that inhibits protein [14] and lipid [15] synthesis, but does not inhibit seed germination. It is suggested that, in these tests, the seeds germinated and the seedlings grew by utilization of stored nutrient reserves, imbibition of water, and cell elongation, none of which was affected by metolachlor. However, when

photoautrophic growth began, protein synthesis was inhibited and seedling weight gain was arrested at that point. Thus, average seedling weights were the same in all concentrations at and above the LOEC.

Seedling Survival and Growth - Metolachlor inhibited survival of *E. crusgalli crusgalli* and *S. alterniflora* in natural and simulated sediments but did not affect survival of *E. crusgalli zelayensis* (Fig. 2). The LOEC for survival in metolachlor was 0.5 mg/kg in natural and simulated sediments with *E. crusgalli crusgalli*; it was 2.5 mg/kg in simulated sediment and 7.5 mg/kg in natural sediment with *S. alterniflora*.

Metolachlor in sediment significantly inhibited growth of seedlings (Fig. 3, Table 3). However, effect of the herbicide with *S. alterniflora* was significantly greater in simulated than in natural sediment. Metolachlor is stable even at pH 1 [15], but is degraded rapidly in aerobic natural soil [15]. It is possible that the microbial flora of the natural saltmarsh sediment contributed to degradation of metolachlor over the 28-d exposure.

Metolachlor is applied to crops as a preemergence herbicide for control of broadleaf and grassy weeds. It is stable in loamy soil for over 64 d [15] and has been detected in surface and groundwaters in the United States [15]. The data suggest that metolachlor could affect germination, survival, and growth of marsh plants when present in water or sediment.

Norflurazon

Seed Germination and Early Growth - Norflurazon did not affect the rate of germination of the species tested. It did reduce the rate of early growth of the two freshwater species (Fig. 4), but the highest concentration, 1 mg/L,

did not affect early growth of *Spartina* (Table 3). The LOEC for norflurazon and *Echinochloa* was 0.05 mg/L. As with metolachlor, average weights of seedlings exposed to norflurazon concentration at and about the LOEC were similar.

Norflurazon is a phenylpyridozinone herbicide that inhibits carotenoid synthesis [16], and because carotenoids protect chlorophyll from degradation by light, norflurazon treatment results in bleached seedlings (Fig. 5). Autotrophic growth of treated seedlings was arrested after initial growth by stored nutrient mobilization, imbibition of water and cell elongation at the LOEC concentration and above, resulting in similar weights.

Seedling Survival and Growth - Norflurazon reduced survival of *E. crusgalli crusgalli* in natural and simulated sediments and of *E. crusgalli zelayensis* in natural sediments (Fig. 6). It did not affect survival of *E. crusgalli zelayensis* in simulated sediment or *S. alterniflora* in either sediment.

The LOEC for growth for norflurazon and *Echinochloa* in both sediments and for *Spartina* in simulated sediment was 0.25 mg/kg (Fig. 7, Table 3). As for metolachlor, effect of norflurazon on average seedling weight of *Spartina* was significantly greater in simulated sediment. Norflurazon is stable under acid conditions [17] but susceptible to degradation by bacteria [18]. As for metolachlor, it is possible that the bacterial flora in the natural saltmarsh soil caused degradation of norflurazon in these tests.

Significance of the Research

There are, at present, no tests that address the problem of effects of contaminated water or sediment on wetland plants. Current toxicity tests with

plants utilize commercial species [19, 20], germination on filter paper [21], or growth substrata that do not simulate natural soils [22,23]. The approach reported here demonstrates that acute exposure of seeds to toxicants in water may inhibit germination and early growth of wetland plants and that chronic exposure of seedlings to toxicants in artificial sediments that are similar to natural sediments may cause death or inhibit growth.

Choice of Test Species

The U.S. Environmental Protection Agency [24] described desirable attributes of organisms for use in toxicity tests with benthic species. The attributes include ecological relevance, variety of endpoints (acute and chronic), all potential routes of exposure should be possible, there should be an adequate amount of tissue for analysis, and ease of organism culture and handling. Also, a plant test species should grow normally in sediments of disparate composition because natural sediments vary widely in composition. The three plants described here satisfy all of these requirements. *Echinochloa* is a widely distributed wetland genus found in North and South America, Europe, Africa, Asia, Australia and the Pacific islands [25]; *Spartina* is often the dominant genus in many marshes of the Atlantic and Gulf Coasts of the United States, and one species, *pectinata*, is found in freshwater of the Northern United States [26]. Acute endpoints are used in the short-term germination and early growth tests and chronic endpoints are used in the survival and seedling growth test. Both tests provide ample tissue for analysis of uptake from water and sediment. Seeds, readily available from suppliers, can be stored in a refrigerator and have a germination rate of approximately 90% [27] and both specieis grow well in

sediments of diverse composition [27]. There is also a large literature on the biology of both species. *Echinochloa crusgalli crusgalli* and *E. crusgalli zelayensis* were shown to be sensitive to industrial and municipal effluents [28]. The effluents inhibited germination, survival, and growth, and when germination and early growth tests were conducted in light and total darkness, effects of toxicants on imbibition of water, cell elongation, utilization of stored nutrients, and photosynthesis were identified.

Choice of Sediments

Composition and structure of sediments are probably the most important factors in substratum toxicity [29], and laboratory use of substances that are not similar to those in which the plant naturally grows may not provide data applicable to field conditions [30, 31]. Grain size [31] and organic content [32, 33, 34] strongly influence the process of equilibrium partitioning of toxicants between sediment particles and pore water. Although natural sediments may be amended in some cases [35], they are often unsuitable for use in toxicity tests because they cannot be duplicated and data from toxicity tests with them must be normalized [30].

Standard sediments are needed for toxicity studies with plants. The standard sediment should be representative of a variety of natural sediments with regard to particle and pore sizes, chemical composition (e.g. quartz vs calcareous), organic content, and nutrient content. Bradshaw [36] gave the qualities of soil required for good plant growth: productive growth, response to fertilizers, good drainage, good water retention, and free of weeds. Others have described the principles of managing man-made soils [37] and procedures for assessment of substances suitable for growing plants [38].

Artificial sediments can be formulated to satisfy the above requirements for plant growth [27]. This report demonstrates that wetland plants responded to herbicides in artificial sediments. In all cases, average seedling weights in simulated sediments were equal to or greater than those in natural sediments. This indicates that the artificial sediments were good growth media and do not contain factors that may inhibit plant growth or confound interpretation of toxicity data.

Conclusions

The wetland plants, *E. crusgalli crusgalli*, *E. crusgalli zelayensis*, and *A. alterniflora*, may be used for evaluation of toxicity of herbicides in water and sediment. Acute (7-d) tests detect effects on early seedling growth; chronic (14- or 28-d) tests detect effects on survival and growth of older seedlings in sediment. Artificial sediments that simulate natural sediments are of value in plant toxicity tests because they support productive growth and allow for assessment of toxic responses. Furthermore, their compositions may be held constant from test to test or may be varied in relation to experimental requirements.

References

[1] Nixon, S.W. in *Estuarine and Wetland Processes*, P. Hamilton and K.B. MacDonald, Eds., Plenum, New York, NY, 1980, pp. 437-524.

[2] Carpenter, J.H. and Huggett, R. in *Concepts in Marine Pollution Measurements*, H.H. White, Ed., University of Maryland, College Park, MD, 1984, pp. 379-403.

[3] *Aquatic Humic Substances: Influence on Fate and Treatment of Pollutants*, I.H. Suffet and P. MacCarthy, Eds., American Chemical Society, Washington, DC, 1989.

[4] Pittinger, C.A., Hand, V.C., Masters, J.A., and Davidson, L.F. in *Aquatic Toxicology and Hazard Assessment (Tenth Volume)*, W.J. Adams, G.A. Chapman, and W.G. Landis, Eds., ASTM STP 971, American Society for Testing and Materials, Philadelphia, PA, 1988, pp. 138-148.

[5] Treshow, M. in *Environmental Toxicology*. J.H. Duffus and J.I. Waddington, Eds., Interim Document 13, World Health Organization, Copenhagen, 1983, pp. 168-184.

[6] Driel, W. van, Smilde, K.W., and Luit, B. van. "Comparison of the Heavy-Metal Uptake of Cyperus esculentus and of Agronomic Plants Grown on Contaminated Dutch Sediments," Miscellaneous Paper D-83-1, Department of the Army, U.S. Army Corps of Engineers, Washington, DC, 1985.

[7] Correll, D.S. and Correll, H.B. *Aquatic and Wetland Plants of Southwestern United States*, Water Pollution Control Research Series 16030 DNL 01/72, U.S. Environmental Protection Agency, Washington, DC, 1972.

[8] Hotchkiss, N. "Common Marsh Plants of the United States and Canada," Resource Publication No. 93, U.S. Department of the Interior, Washington, DC, 1970.

[9] Seneca, E.D., *American Journal of Botany*, Vol. 61, 1974, pp. 947-956.

[10] Folk, R.L. *Petrology of Sedimentary Rocks*, Hemphill Publishing Co., Austin, TX, 1980.

[11] Hoagland, D.R. and Arnon, D.I. "The Water Culture Method for Growing Plants Without Soil," Circular 347, California Experiment Station, Berkeley, CA, 1950.

[12] U.S. Department of Agriculture, "Soil Survey Laboratory Methods and Procedures for Collecting Soil Samples," Soil Survey Investigations Report No. 1, Soil Conservation Service, Washington, DC, 1972.

[13] *SAS User's Guide: Statistics, 1982 Edition.* SAS Institute, Inc., Cary, NC, 1982.

[14] Jaworski, E.G. in *Herbicides: Chemistry, Degradation and Mode of Action*, P.C. Kearney and D.D. Kaufman, Eds. Dekker, New York, NY, 1975, Chapt. 6, pp. 349-376.

[15] U.S. Environmental Protection Agency, "Pesticide Fact Sheet, Metolachlor," Fact Sheet No. 106, Office of Pesticides and Toxic Substances, Washington, DC, 1987, 9 pp.

[16] Sandman, G. and Boger, P. in *Biochemical Responses Induced by Herbicides*, D.E. Moreland, J.B. St. John, and F.D. Hess, Eds. ACS Symposium Series 181, American Chemical Society, Washington, DC, 1982, Chapt. 7, pp. 111-130.

[17] *The Pesticide Manual: a World Compendium, 6th ed.*, C.R. Worthing, Ed. British Crop Protection Council, London, 1979.

[18] *Herbicides Handbook, 4th ed.* Weed Science Society of America, 1979.

[19] U.S. Environmental Protection Agency. "Seed Germination/Root Elongation Toxicity Test," EG12, Office of Toxic Substances, Office of Pesticides, Washington, DC, 1982.

[20] OECD "Terrestrial Plants, Growth Test, OECD Guideline for Testing of Chemicals, No. 208," Organization for Economic Cooperation and Development, Paris, 1984.

[21] Wang, W., *Environmental Toxicology and Chemistry*, Vol. 6., 1987, pp. 409-414.

[22] Lee, C.R., Sturgis, T.C., and Landin, M.C., *Journal of Plant Nutrition*, Vol. 3, 1981, pp. 139-151.

[23] Azpiazu, M.N., Romero, E., and Diaz, J.M., *Water, Air, and Soil Pollution*, Vol. 28, 1986, pp. 1-26.

[24] U.S. Environmental Protection Agency. *Issues Related to the Assessment and Resolution of Problems Associated With Contaminated Sediment*, Office of Water, Office of Water Regulations and Standards, Washington, DC, 1989.

[25]Holm, L.G., Plucknett, D.L., Pancho, J.W., and Herberger, J.P., *The World's Worst Weeds*. The University of Hawaii Press, Honolulu, HI, 1972.

[26] Muenscher, W.C., *Aquatic Plants of the United States*. Comstock Publishing Associates, Cornell University Press, Ithaca, NY, 1944.

[27] Walsh, G.E., Weber, D.E., Brashers, L.K., and Simon, T.L., *Environmental and Experimental Botany*, in press.

[28] Walsh, G.E., Weber, D.E., Simon, T.L. and Brashers, L.K., Environmental Toxicity and Chemistry, in press.

[29] LeBaron, H.M. in *Residue Reviews, Vol. 32, The Triazine Herbicides*. F.A. Gunther and J.D. Gunther, Eds. Springer-Verlag, New York, NY, 1970, pp. 311-353.

[30] van Straalen, N.M. and Denneman, C.A., *Ecotoxicology and Environmental Safety*, Vol. 18, 1989, pp. 241-251.

[31] Spies, R.B., *Marine Environmental Research*, Vol. 27, 1989, pp. 73-75.

[32] Stackhouse, R.A. and Benson, W.H., *Aquatic Toxicology*, Vol. 13, 1988, pp. 99-108.

[33] Stevenson, F.J., *BioScience*, Vol. 22, 1972, pp. 643-650.

[34] Swindoll, C.M. and Applehans, F.M., *Bulletin of Environmental Contamination and Toxicology*, Vol. 39, 1987, pp. 1055-1062.

[35] Barko, J.W. and Smart, R.M., *Journal of Ecology*, Vol, 71, 1983, pp. 161-175.

[36] Bradshaw, A.D., *Soil Use and Management*, Vol. 5, 1989, pp. 101-108.

[37] Stewart, V.I. and Scullion, J., *Soil Use and Management*, Vol. 5, 1989, pp. 109-116.

[38] Adams, W.A., Stewart, V.I., and Thornton, D.J., *Journal of the Sports Turf Research Institute*, Vol. 47, 1971, pp. 77-85.

TABLE 1 - *Composition of natural and simulated freshwater and saltmarsh sediments.*

Class	Particle Size μm	% Composition, by weight Freshwater	Saltmarsh
Coarse sand	500 - 1500	0.6	33.6
Medium sand	250 - 499	9.5	58.8
Fine sand	63 - 249	67.4	4.9
Silt	4 - 62	10.3	0.6
Clay	< 4	6.7	0.7
Organic	-	4.9	0.8
Lost during analysis	-	0.6	0.6

TABLE 2 - *pH and cation exchange capacity (CEC) of freshwater and estuarine sediments used in toxicity tests with herbicides.*

	Freshwater		Estuarine	
	Natural	Simulated	Natural	Simulated
pH	5.8	7.5	2.9	7.4
CEC (meq/100g)	16.6	19.0	14.1	19.0

TABLE 3 - *LOECs for growth of wetland plants exposed to herbicides in water and natural and simulated sediments.*

	Water, mg/L	Sediment, mg/kg	
		Natural	Simulated
Metolachlor			
E. crusgalli crusgalli	0.25	0.25	0.25
E. crusgalli zelayensis	0.25	0.10	0.25
S. alterniflora	0.50	10.1	0.50
Norflurazon			
E. crusgalli crusgalli	0.05	0.25	0.25
E. crusgalli zelayensis	0.05	0.25	0.25
S. alterniflora	> 1	> 2	0.25

FIG. 1 - *Average seedling weight of Echinochloa crusgalli crusgalli* (A), *Echinochloa crusgalli zelayensis* (B), *and Spartina alterniflora* (C) *exposed to metolachlor in water.* * - *significantly lower than control*, $\alpha = 0.05$; C = *control.*

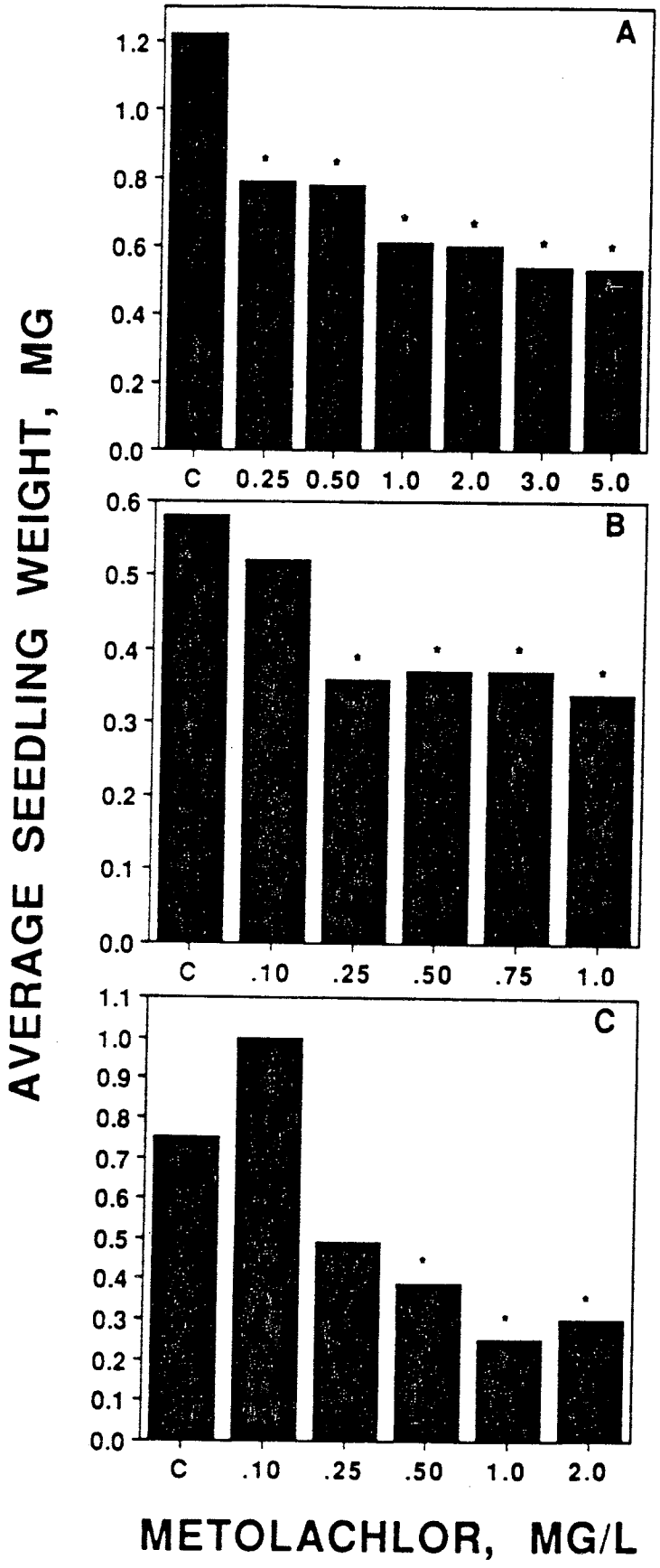

A
1.2
1.0
0.8
0.6
0.4
0.2
0.0
C
0.25
0.50
1.0
2.0
3.0
5.0
B
0.6
0.5
0.4
0.3
0.2
0.1
0.0
C
.10
.25
.50
.75
1.0
C
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
C
.10
.25
.50
1.0
2.0
AVERAGE SEEDLING WEIGHT, MG
METOLACHLOR, MG/L

FIG. 2 - *Survival of Echinochloa crusgalli crusgalli* (A), *Echinochloa crusgalli zelayensis* (B), *and Spartina alterniflora* (C) *in natural and simulated sediments contaminated with metolachlor.* * - *significantly lower than control*, $\alpha = 0.05$; C = *control.*

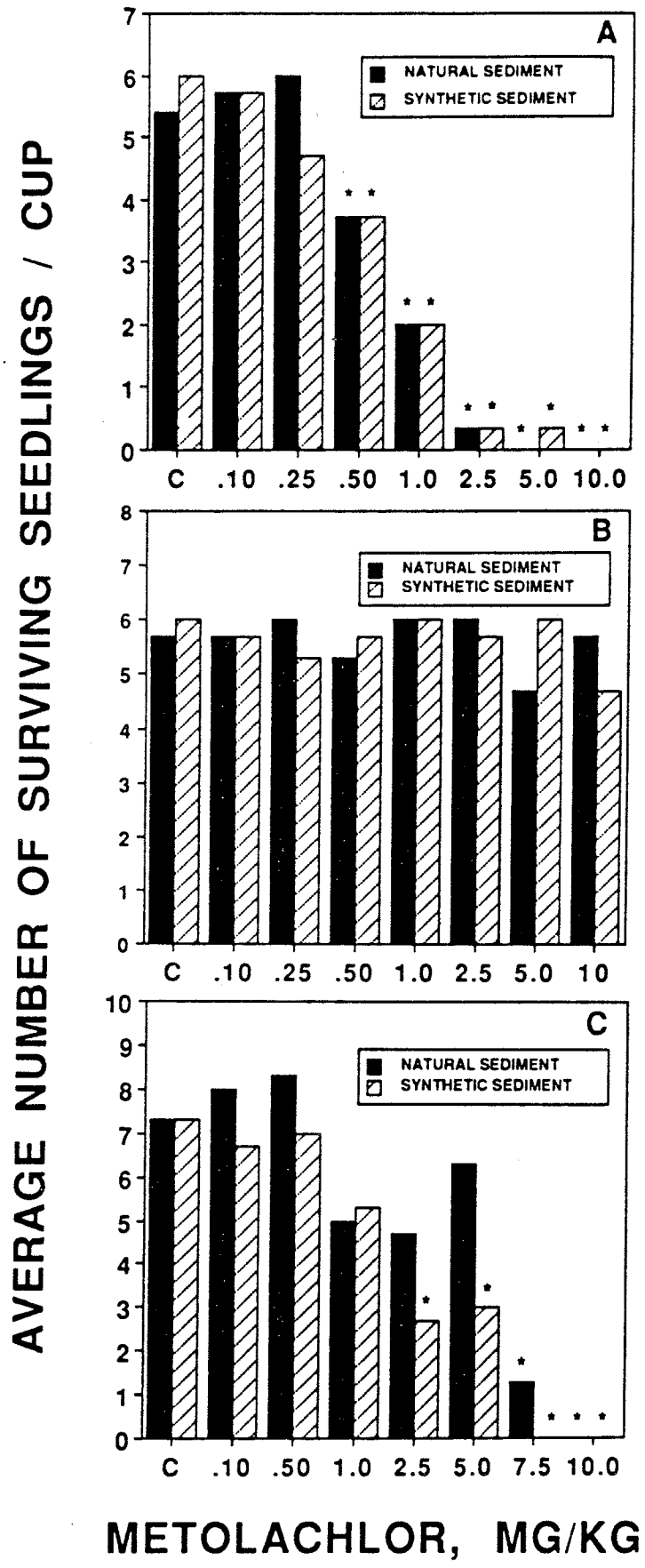

A
NATURAL SEDIMENT
SYNTHETIC SEDIMENT
C .10 .25 .50 1.0 2.5 5.0 10.0
B
NATURAL SEDIMENT
SYNTHETIC SEDIMENT
C .10 .25 .50 1.0 2.5 5.0 10
C
NATURAL SEDIMENT
SYNTHETIC SEDIMENT
C .10 .50 1.0 2.5 5.0 7.5 10.0
AVERAGE NUMBER OF SURVIVING SEEDLINGS / CUP
METOLACHLOR, MG/KG

FIG. 3 - *Average weights of seedlings of Echinochloa crusgalli crusgalli* (A), *Echinochloa crusgalli zelayensis* (B), *and Spartina alterniflora* (C), *exposed to metolachlor in natural and simulated sediments.* * - *significantly lower than control*, $\alpha = 0.05$; C = *control.*

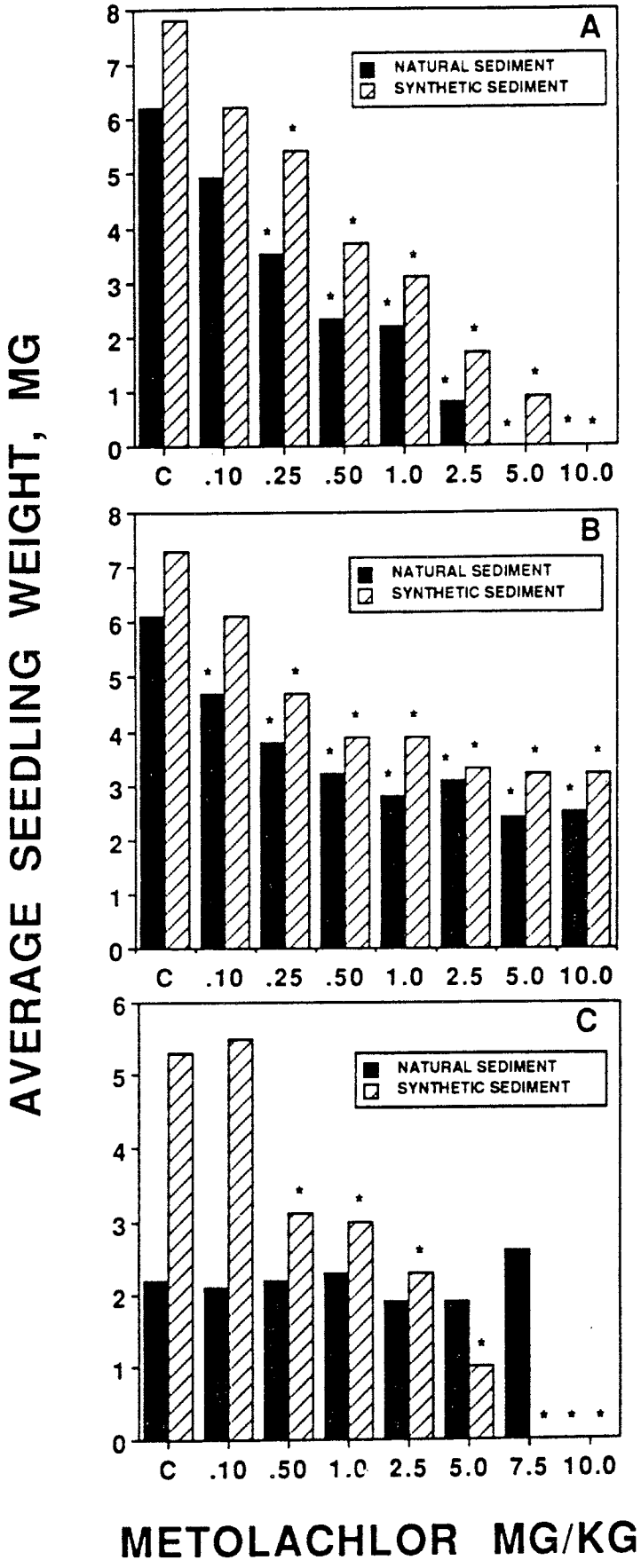

A
NATURAL SEDIMENT
SYNTHETIC SEDIMENT
0 1 2 3 4 5 6 7 8
C .10 .25 .50 1.0 2.5 5.0 10.0
B
NATURAL SEDIMENT
SYNTHETIC SEDIMENT
0 1 2 3 4 5 6 7 8
C .10 .25 .50 1.0 2.5 5.0 10.0
C
NATURAL SEDIMENT
SYNTHETIC SEDIMENT
0 1 2 3 4 5 6
C .10 .50 1.0 2.5 5.0 7.5 10.0
AVERAGE SEEDLING WEIGHT, MG
METOLACHLOR MG/KG

FIG. 4 - *Average weights of seedlings of Echinochloa crusgalli crusgalli* (A), *and Echinochloa crusgalli zelayensis* (B), *exposed to norflurazon in water.* * - *significantly lower than control*, $\alpha = 0.05$; C = *control.*

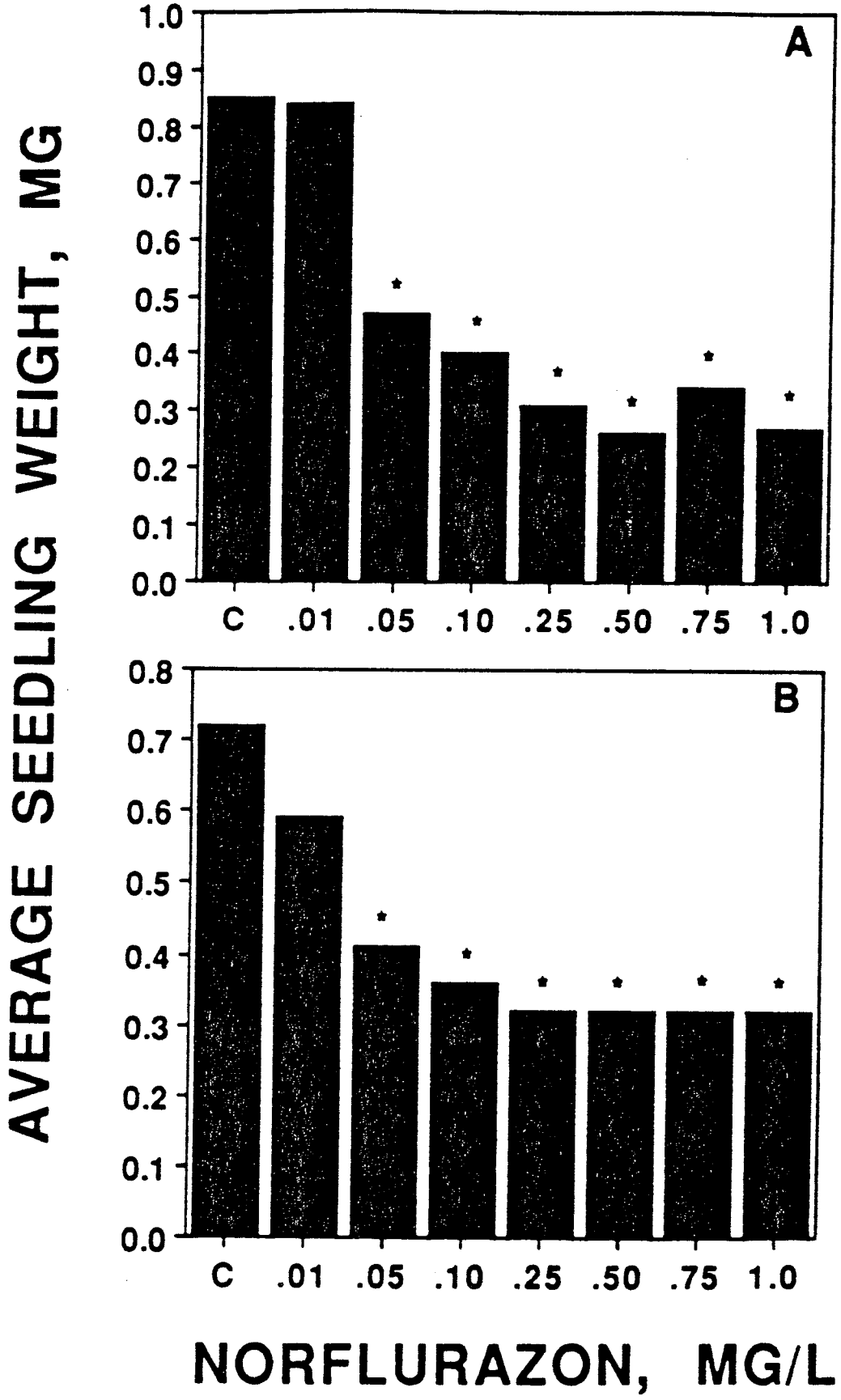

AVERAGE SEEDLING WEIGHT, MG
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
A
C
.01
.05
.10
.25
.50
.75
1.0
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
B
C
.01
.05
.10
.25
.50
.75
1.0
NORFLURAZON, MG/L

FIG. 5 - Bleaching of *Echinochloa crusgalli crusgalli by norflurazon in water.* A - *control*, B - 0.05 *mg/L*, C - 0.25 *mg/L*.

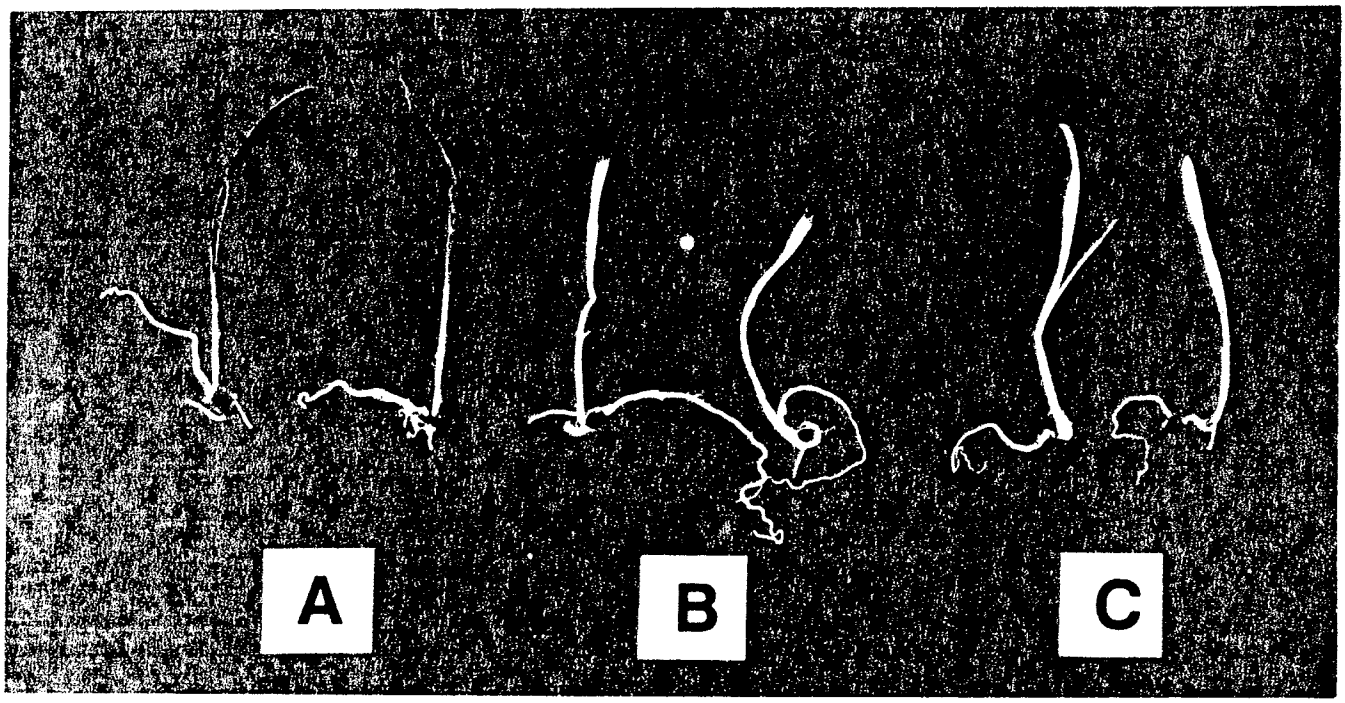

A
B
C

FIG. 6 - *Survival of Echinochloa crusgalli crusgalli* (A) *and Echinochloa crusgalli zelayensis* (B) *in natural and simulated sediments contaminated with norflurazon.* * - *significantly lower than control*, $\alpha = 0.05$; C = *control.*

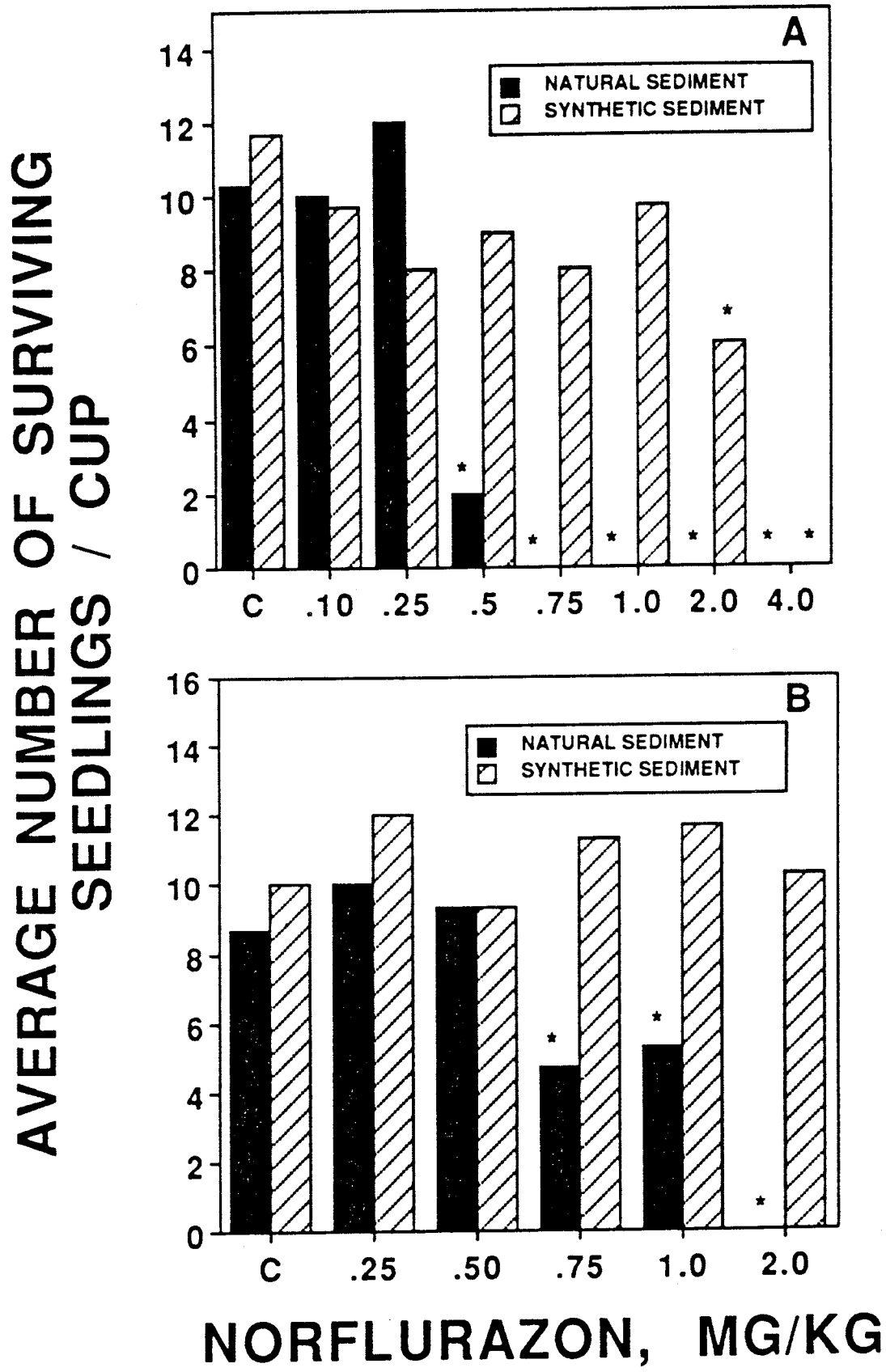
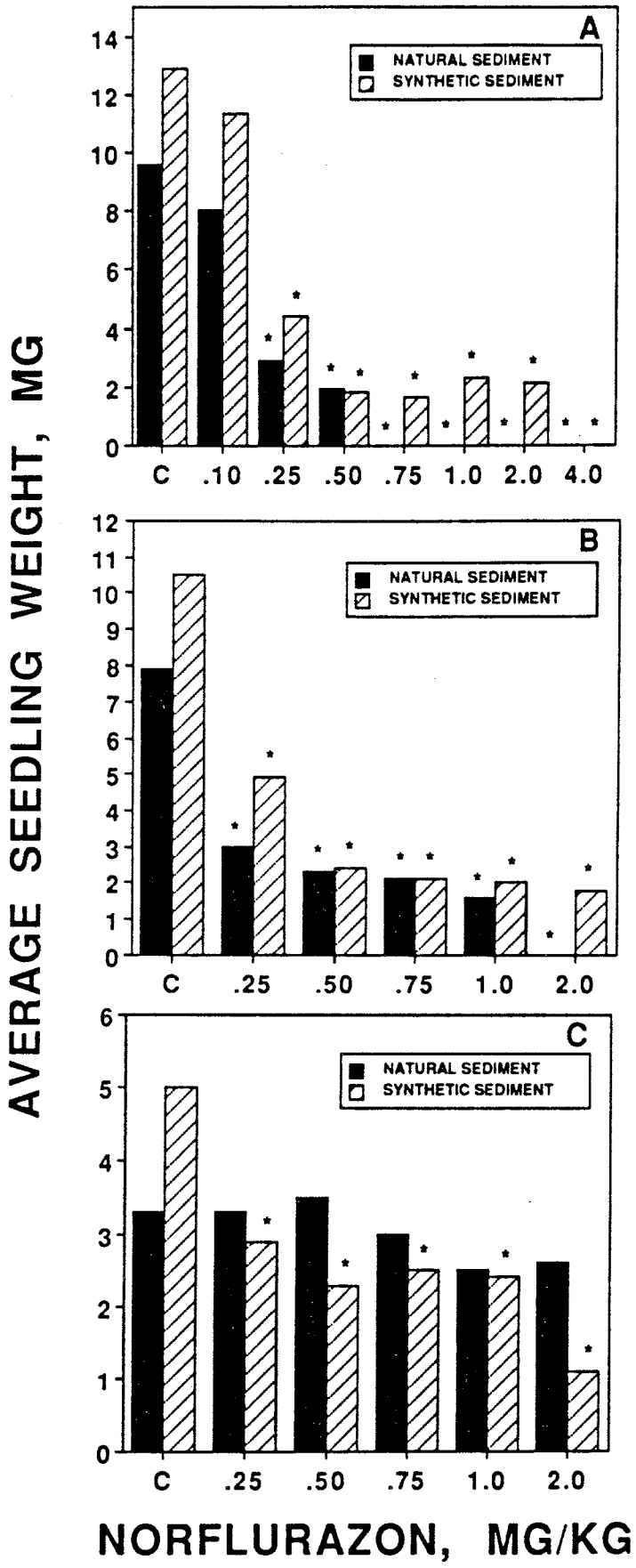

AVERAGE NUMBER OF SURVIVING SEEDLINGS / CUP
A
NATURAL SEDIMENT
SYNTHETIC SEDIMENT
14
12
10
8
6
4
2
0
C
.10
.25
.5
.75
1.0
2.0
4.0
B
NATURAL SEDIMENT
SYNTHETIC SEDIMENT
16
14
12
10
8
6
4
2
0
C
.25
.50
.75
1.0
2.0
NORFLURAZON, MG/KG

FIG. 7 - *Average weights of seedlings of Echinochloa crusgalli crusgalli* (A), *Echinochloa crusgalli zelayensis* (B), *and Spartina alterniflora* (C), *exposed to norflurazon in sediments.* * - *significantly lower than control,* α = 0.05; C = *control.*


A
NATURAL SEDIMENT
SYNTHETIC SEDIMENT
14
12
10
8
6
4
2
0
C .10 .25 .50 .75 1.0 2.0 4.0
B
NATURAL SEDIMENT
SYNTHETIC SEDIMENT
12
11
10
9
8
7
6
5
4
3
2
1
0
C .25 .50 .75 1.0 2.0
C
NATURAL SEDIMENT
SYNTHETIC SEDIMENT
6
5
4
3
2
1
0
C .25 .50 .75 1.0 2.0
AVERAGE SEEDLING WEIGHT, MG
NORFLURAZON, MG/KG

APPENDIX 3

RESPONSES OF WETLAND PLANTS TO EFFLUENTS IN WATER AND SEDIMENT

RESPONSES OF WETLAND PLANTS TO EFFLUENTS IN WATER AND SEDIMENT[1]

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Walsh, G.E., Weber, D.E, Nguyen, M.T. and Esry, L.K. Responses of wetland plants to toxicants in water and sediments. Environmental and Experimental Botany, *in press*. Responses of two wetland vascular plants, Echinochloa crusgalli and Sesbania macrocarpa, exposed to effluents from a coke plant, a pulp mill, and a wastewater treatment plant were measured in three types of tests: seed germination and early growth, seedling survival and growth in hydroponic culture, and seedling survival and growth in sand and synthetic sediments with sand, clay, silt, and 3, 5, 7.5, or 10% organic contents. There was no effect of effluents on germination. Growth rates were reduced significantly in all tests except for E. crusgalli exposed to effluent from a wastewater treatment plant in sediment. There, the effluent stimulated growth. Increasing concentrations of organic matter in sediments had little effect on toxicity of effluents.

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[1] Contribution No. 712, United States Environmental Protection Agency, Gulf Breeze, FL, U.S.A. Mention of trade names or commercial products in this report does not constitute endorsement by the U.S. Environmental Protection Agency.

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INTRODUCTION

Several laboratory tests have been recommended for use in toxicity studies with vascular plants. Measured responses in such tests include rates of germination,[1, 15, 20, 21] seedling survival,[10] root elongation,[13, 19, 20, 21] and growth of entire plants.[5] Tests have been performed in hydroponic systems,[2,7] on filter paper,[12] in sand,[9,12] and in synthetic growth media designed to be similar to natural soils and sediments.[17,18] Interpretation of toxicity data from the various types of tests with regard to possible effects in the field is difficult, i e., tests on germination and root growth on filter paper are satisfactory for detection of plant toxicants, but relevance of results to complicated soil or sediment systems is dubious.[13] In the studies reported here, responses of two wetland plant species to effluents from a coke plant, a pulp mill, and a wastewater treatment plant are compared in germination and early seedling growth, hydroponic, and sediment tests. The studies were performed to determine how method of exposure and composition of sediment affect toxicity of effluents to wetland plants because wetlands may accumulate toxicants.[17,18]

METHODS

Plant species

Test species were the common wetland plants, Echinochloa crusgalli (L.) Palisot de Beauvois var. crusgalli (Poaceae) and Sesbania macrocarpa Muhl. ex Raf. (Leguminosae). Plant names were confirmed by examination of flowers and seeds.[3,4] Seeds were obtained from Wildlife Nurseries, Oshkosh, WI 54903-2724, and stored dry at 4°C. Germination percentages confirmed in our laboratory were >90% for E. crusgalli and approximately 60% for S. macrocarpa.

Hydroponic and sediment tests were conducted with 5-d old seedlings. Before tests were started, seeds were treated to remove possible pathogenic organisms and to break dormancy. Seeds of E. crusgalli were immersed in 1% sodium hypochlorite solution for 20 min and rinsed with deionized water. Seeds of S. macrocarpa were immersed in concentrated sulfuric acid for 30 min, rinsed, and soaked overnight in deionized water. Treated seeds were planted in coarse sand wetted with deionized water and incubated at 25 ± 1°C under 110 $\mu Em^{-2}/sec^{-1}$ cool white fluorescent light on a 16 hr light: 8 hr darkness cycle. These conditions of temperature and lighting were also used in all of the tests.

Synthetic sediments

Synthetic sediments were used in these tests in order to control the relative contents of sand, silt, clay and particulate and dissolved organic matter (Table 1). The sediments are identified by organic content. Sand and particulate organic matter composed of sphagnum moss, and cow manure/compost were washed thoroughly in deionized water and dried before use. The dry moss and cow manure/compost were ground to an average particle size of 840 μm.

Dry components were mixed in the desired relative amounts and mixed with full strength Hoagland nutrient solution,[6] or undiluted effluent to which full strength Hoagland nutrients were added. The ratio was 42 ml solution to 135 g sediment. This produced a wet sediment that, when placed in a styrofoam cup 5.5 cm high x 7.4 cm diameter, was covered by approximately 0.5 cm of solution. Wet sediments were kept in the laboratory for 24 hr at room temperature for stabilization of pH.

Four synthetic formulations with 3, 5, 7.5 and 10% organic content were characterized (Table 2) and used in the sediment toxicity test.

The following methods were used for characterization of wet sediments: pH was measured with a Phi 12 pH/ISE meter,[8] Eh with a Radiometer/Copenhagen PHM pH/Eh meter,[8] total organic matter by ashing at 550°C for 24 hr,[8] and all other characteristics given in Table 2 by USDA methods.[16]

Effluents

Single grab samples of effluents were shipped under ice in insulated containers from a coke plant, a pulp mill, and a wastewater treatment plant and were cool to the touch when received at the laboratory less than 24 hr after collection. Sub samples were taken immediately upon receipt for the germination and early growth test and analysis of pH (Beckman Phi 12 pH/ISE meter) and salinity (American Optical Co. No. 10419 temperature - compensated refractometer). The effluents were stored in darkness at 4°C and used in the hydroponic and sediment tests within 24 hr of receipt.

Seed germination and early seedling growth

The seed germination and early seedling growth test was conducted with E. crusgalli following methods described by Walsh et al.[17,18] Effluents were not filtered and were diluted to 1, 10, 25, 50, and 75% with deionized water. Tests with deionized water as the control and with undiluted effluent (100%) were also conducted. Approximately 8 ml of each solution were added to each of six polystyrene Petri dishes, 47 mm in diameter and with tightly fitting lids (Millipore Filter Corp., Bedford, MA). Ten seeds were placed in each dish and three dishes of each treatment were incubated under the light regime given above and in total darkness at 25±1°C. Germinated seeds were counted each day for 7 days, at which time the seedlings were harvested and dried for 24 hr at 103°C. After drying, the seedlings were weighed to the nearest 0.1 mg and the average weight for each group was calculated.

Survival and growth in hydroponic culture

Seedlings of E. crusgalli and S. macrocarpa were removed carefully from sand culture and placed in 125-ml Erlenmeyer flasks wrapped with aluminum foil to prevent photodecomposition of toxicants and growth of algae. The base of each seedling was wrapped with cotton just above the roots, and the roots immersed in test solution. This was full strength Hoagland nutrient solution (control), effluent to which Hoagland nutrients were added and then diluted with control nutrient solution to 1, 10, 25, and 50%, and undiluted effluent with Hoagland nutrients. One seedling was exposed in each flask, and six seedlings were exposed to each test liquid. The liquid in each flask was replaced with fresh exposure solution after 7 d. The hydroponic test was conducted for 14 d, at which time survival was determined and the seedlings harvested. At harvest, surviving seedlings were rinsed with deionized water, divided into roots and shoots, dried for 24 hr at 103°C, and weighed to the nearest 0.1 mg. Average seedling weight for each control and treated group was calculated.

Survival and growth in sediments

Tests in sediment were conducted by the method of Walsh et al.[17,18] Seedlings were removed carefully from sand culture and transplanted to sediments in cups. Ten seedlings of E. crusgalli or 3 seedlings of S. macrocarpa were planted in each of 3 cups that contained sediment treated with full strength Hoagland nutrient solution (control), or undiluted effluent with Hoagland nutrients. As a reference, they were also planted in similar cups of washed sand. Seedlings were watered each Monday, Wednesday, and Friday with 20 ml of one-half strength nutrient solution or effluent containing nutrients in the first week and 40 ml in the second week. After exposure for 2 weeks, seedlings were examined for survival, harvested, rinsed with deionized water, separated into roots and shoots, and dried and weighed as above.

Data analysis

The EC50 was calculated by straight-line graphical interpolation.[1]

Differences between treatment means were evaluated by two-way analysis of variance (ANOVA).[15] Means of treated groups were compared with control means by the Duncan's multiple range procedure when F values were significant (P = 0.05).[15]

RESULTS

Coke plant effluent

Seed germination and early seedling growth. Coke plant effluent (pH 6.4, salinity 2 $^{0}/_{00}$ did not affect germination of E. crusgalli in either light or darkness after 7 d of exposure. It did, however, cause reduction in average seedling weight in light and darkness (Table 3). Effect was greater in light, where the lowest observed effect concentration (LOEC) was 10% effluent. The LOEC in darkness was 50% effluent. The EC50s were 35% effluent (light) and 96% effluent (darkness).

Hydroponic test. Coke plant effluent did not affect survival of E. crusgalli or S. macrocarpa in the hydroponic test.

The effluent inhibited growth of roots, shoots, and entire seedlings (root weight + shoot weight) of E. crusgalli (Table 4). The LOEC for each was 25% effluent, and EC50s were 16% (roots), 18% (shoots) and 17% (entire seedlings). Although the LOEC was lower in the seed germination and early growth test in light, the EC50s were lower in the hydroponic test here and in the tests given below.

Results with S. macrocarpa (Table 4) were similar to those with E. crusgalli. All had the same LOEC, 25% effluent. EC50s were: 53% (roots), 56% (shoots), and 55% (entire seedlings). Echinochloa crusgalli was generally more sensitive than S. macrocarpa in tests with effluents.

Sediment test. Coke plant effluent did not inhibit survival, but it did inhibit growth of both species in sediments. Growth of roots, shoots, and entire seedlings of E. crusgalli was inhibited significantly in sand and all sediments (Fig. 1A). There was no difference in dry-weight reduction among sand and the 4 synthetic sediments with different organic contents. Inhibition of growth of roots, shoots, and entire seedlings of S. macrocarpa (Fig. 1B) was significant in sand and synthetic sediment of 3% organic content but not in sediments of higher organic content when paired comparisons were made between weights of each control and each group of treated roots, shoots, and entire seedlings. However, all weights of effluent-treated seedlings were lower than the weights of comparable controls. In a paired comparison of total weights of control vs. treatments, weights of roots, shoots, and entire seedlings were significantly lower in effluent exposures than in controls ($P = 0.0001$ for each paired group).

Pulp mill effluent

Seed germination and early seedling growth. Effluent from the pulp mill (pH 7.3, salinity 2 $^{o}/_{oo}$) did not affect germination of seeds of E. crusgalli. It inhibited growth of seedlings in light and darkness at high concentrations (Table 3). The LOEC in light was 75% and the EC50 was 86% effluent; the LOEC was 50% and the EC50 was 84% in darkness.

Hydroponic test. The data above suggest that pulp mill effluent was not highly toxic to E. crusgalli. However, although there was no significant effect on survival of E. crusgalli or

S. macrocarpa in the hydroponic test, the lowest concentration used, 1% effluent, caused significant inhibition of growth of E. crusgalli (Table 4), with EC50s of approximately 1% for roots, 2% for shoots, and 1% for entire seedlings. Although there was an apparent trend toward lower root weights of S. macrocarpa with increasing effluent concentration (Table 4), differences from the control were not statistically significant except for shoots at high concentrations.

Sediment test. Pulp mill effluent did not affect survival of E. crusgalli or S. macrocarpa. The effluent did inhibit growth of both species but in different ways. There was significant inhibition of E. crusgalli root growth at 5, 7.5 and 10% organic content and of shoots and entire seedlings at 10% organic content (Fig. 2A). In contrast, significant inhibition of roots, shoots, and entire seedlings of S. macrocarpa occurred only in sand (Fig. 2B).

Wastewater treatment plant effluent

Seed germination and early seedling growth. As with the other effluents, there was no effect of wastewater treatment plant effluent (pH 6.9, salinity 0 $^{o}/_{oo}$) on germination of E. crusgalli, but growth was inhibited significantly (Table 3) in light (LOEC = 25%, EC50 = 32% effluent) and darkness (LOEC = 10%, EC50 = 50% effluent).

Hydroponic test. There was no significant effect of wastewater treatment plant effluent on survival of either species in the hydroponic test, but growth was inhibited. Echinochloa crusgalli was more sensitive in this test than S. macrocarpa (Table 4). The LOECs for roots, shoots and entire seedlings for E. crusgalli was 1% effluent and 25% for S. macrocarpa. The EC50s for E. crusgalli were <1% (roots) and 3% effluent (shoots and entire seedlings). No EC50s could be calculated

for S. macrocarpa because growth inhibition was less than 50%; maximum growth inhibition was 39.7% of the control.

Sediment test. Wastewater treatment plant effluent had no effect upon survival of E. crusgalli or S. macrocarpa in the sediment test. Comparison of each control with each treated sample within each group (sand, 3% organic, etc.) did not reveal statistically significant differences in root, shoot, or entire seedling weights of E. crusgalli (Fig. 3A). However, because treated shoot and entire seedling weights were always greater than controls, paired comparisons were made between all controls vs. all treated groups. This comparison showed that the effluent caused significant increases in weights of shoots ($P = 0.0001$) and entire seedlings ($P = 0.0031$) but not of roots ($P = 0.7529$).

This was not true for S. macrocarpa (Fig. 3B). Whereas paired comparisons between control and treated samples within each group did not reveal significant differences, treated group values were always lower than control groups. Paired comparison of all control groups vs. all treated groups showed significant weight reductions of roots ($P = 0.0058$) shoots ($P = 0.0132$) and entire seedlings ($P = 0.0064$).

DISCUSSION

None of the effluents affected germination of E. crusgalli, and this was of no value for assessment of toxic effects in these tests. However, Walsh et al.[18] reported that effluents from a sewage treatment plant and a coke plant (not the one reported here) did inhibit the rate of germination of E. crusgalli, so germination may detect toxicity in some cases.

None of the effluents affected survival in any test. These results were similar to those of Walsh et al.,[18] who reported that sewage treatment plant, textile mill, pulp and paper mill, metals plating works, coke plant, and tannery effluents did not affect survival of two varieties of E. crusgalli in sediment. As was found in germination tests, survival of seedlings was not a sensitive end-point for toxicity tests with wetland plants.

Seedling growth was a sensitive criterion of toxicity in these studies. All effluents inhibited growth in light and darkness. The LOECs for growth of early seedlings in pulp mill and wastewater treatment plant effluents were lower in darkness than in light, whereas in coke plant effluent they were lower in light than in darkness. This suggests that effluents may inhibit processes associated with autotrophic growth such as utilization of seed nutrients or imbibition of water. They may also inhibit chemical processes associated with photosynthesis. It is also possible that toxicants may be photo-oxidized and thus exert greater effect in darkness.

The hydroponic test was more sensitive than the seed germination and early seedling growth test with E. crusgalli in all effluents, perhaps because exposure time was longer and the toxic substances were shielded from light. Also, E. crusgalli was more sensitive than S. macrocarpa in

pulp mill and wastewater treatment plant effluents, but sensitivity was approximately the same in the coke plant effluent. Relative sensitivity of test species would seem to be an important consideration in toxicity tests with plants.

It is clear that growth of vascular wetland plants is inhibited by effluents in water and that effects may be detected in several types of laboratory tests. It is more likely, however, that plants are exposed through their roots to toxicants in soil or sediment and that the toxicants must be absorbed by roots and translocated to other plant organs. The tests with sand and synthetic organic sediments were done to determine if sediments may attenuate or potentiate effects of bioactive substances. It is well known that soils and sediments may attenuate or even eliminate effects of toxicants.[14] The presence of organic matter in increasing concentrations did not stop the effluents from being toxic and, in the case of E. crusgalli and pulp mill effluent, toxicity was greatest in sediment with the highest concentration of organic matter.

It is suggested that germination and hydroponic growth tests may be used for detection of plant toxicants, but that they should be combined with plant growth tests in sediments for estimation of possible effects in the field.

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REFERENCES

1. APHA (1989) Standard Methods for the Examination of Water and Wastewater 17th ed. American Public Health Association, Washington, D.C.
2. Bertelsen F. and Gissel - Nielsen G. (1987) Toxicity of root-applied sulphate in Zea mays. Environ. Geochem. Health 9, 12-16.
3. Clewell A.F. (1985) Guide to the Vascular Plants of the Florida Panhandle. University Presses of Florida, Florida State University Press, Tallahassee, FL.
4. Correll D.S. and Correll H.B. (1972) Aquatic and Wetland Plants of Southwestern United States. Water Pollution Control Research Series 16030 DNL 01/72. U.S. Environmental Protection Agency, Washington, D.C.
5. Haley J.E. and Fermanian T.W. (1989) Flurprimidol effect on the emergence and growth of annual bluegrass and creeping bentgrass. Agron. J. 81, 198-202.
6. Hoagland D.R. and Arnon D.I. (1950) The Water Culture Method for Growing Plants Without Soil. Circular 347, California Experiment Station, Berkeley, CA.
7. Islam A.K.M.S., Asher C.J. and Edwards D.G. (1987) Response of plants to calcium concentrations in flowing solution culture with chloride or sulphate as the counter-ion. Plant Soil 98, 377-395.
8. Jackson M.L. (1960) Soil Chemical Analyses. Prentice Hall, Englewood Cliffs, NJ.
9. Lee C.R., Sturgis T.C. and Landin M.C. (1987) Heavy metal uptake by marsh plants in hydroponic solution cultures. J. Plant Nut. 3, 139-151.
10. Miyamoto S. (1989) Salt effects on germination, emergence, and seedling mortality of onion. Agron. J. 81, 202-207.
11. Mrozak E., Jr. and Funicelli N.A. (1982) Effect of zinc and lead on germination of Spartina alterniflora Loisel seeds at various salinities. Environ. Exptl. Bot. 22, 23-32.

12. Paganelli D.J., Seiler J.R. and Feret P.P. (1987) Root regeneration as an indicator of aluminum toxicity in loblolly pine. Plant Soil 102, 115-118.

13. Ratsch H.C. and Johndro D. (1986) Comparative toxicity of six test chemicals to lettuce using two root elongation test methods. Environ. Monitor. Assess. 6, 267-276.

14. Sabljic A. (1989) Quantitative modeling of soil sorption for xenobiotic chemicals. Environ. Health Perspect. 83, 179-190.

15. SAS Institute, Inc. (1989) SAS/STAT User's Guide: Version 6, 4th ed., Vol. 1. SAS Institute, Inc., Cary, NC.

16. USDA/SCS (1984) Procedures for Collecting Soil Samples and Methods of Analysis for Soil Survey. U.S. Department of Agriculture, Soil Conservation Service. Soil Survey Investigations Report No. 1. Washington, D.C.

17. Walsh G.E., Weber D.E., Simon T.L. and Brashers L.K. Effects of effluents on germination, survival, and growth of marsh plants in water and sediment. Environ. Toxicol. Chem. 10, 517-525.

18. Walsh G.E., Weber D.E., Simon T.L., Brashers L.K. and Moore J.C. Use of marsh plants for toxicity testing of water and sediment in J. Gorsuch and W. Wang, eds. Second Symposium on Use of Plants for Toxicity Assessment. American Society for Testing and Materials, Philadelphia, PA. in press.

19. Wang W. (1987) Root elongation method for toxicity testing of organic and inorganic pollutants. Environ. Toxicol. Chem. 6, 409-414.

20. Wang W. (1989) Determination and reduction of phytotoxicity of two industrial waste effluents. Water Air Soil Pollut. 44, 303-373.

21. Wang W. (1989) Toxicity assessment of the aquatic environment using phytoassay methods. Illinois State Water Survey Division, SWS Contract Report 455, Peoria, IL.

Table 1. Compositions of synthetic sediments used in toxicity tests with Echinochloa crusgalli and Sesbania exaltata. 0.01 g of humic acids was added to each synthetic sediment

	Composition, % by Weight			
Components*	3% Organic	5% Organic	7.5% Organic	10% Organic
Sand	82.8	81.1	76.7	72.5
Clay and silt	14.6	14.3	13.6	12.8
Dolomite	0.5	0.5	0.5	0.4
Sphagnum moss	2.1	2.1	2.0	1.9
Cow manure/compost	-	2.0	7.2	12.4

*,Suppliers, Medium sand: Mystic White No. 45, New England Silica, Inc., South Windsor, CN; Clay and silt: ASP 400, Englehard Corp., Edison, NJ; Dolomite: Southern Agri-Minerals Corp., Hartford, AL; Sphagnum moss: Hyde Park Products, Inc., Mamroneck, NY; Cow manure/compost: Hyponox Corp., Atlanta, GA; Humic acids: Aldrich Chemical Co., Milwaukee, WI.

Table 2. Characterization of synthetic sediments used in toxicity tests with wetland plants

Substratum (% organic)	Percentage Sand (mm) 1-2	.5-1	.25-.5	.1-.25	.05-.1	Silt (mm) .002-.05	Clay (mm) <.002
3	0	13.4	63.8	5.6	0.6	12.1	4.5
5	0	13.8	62.0	5.4	0.6	13.2	5.0
7.5	0.2	13.8	58.2	5.8	0.6	16.1	5.5
10	0	12.8	56.0	6.6	0.8	20.0	3.8

	Cond. (mS/cm)	Extract. bases (meq/100g)	Extract. acids (meq/100g)	pH	Eh	CEC (meq/100g)
3	0.17	4.86	0.16	6.4	462	5.1
5	0.37	7.77	0.52	6.8	412	8.3
7.5	0.83	17.16	1.74	6.9	373	18.9
10	1.33	25.96	2.07	7.2	347	28.0

Table 3. Inhibition of Echinochloa crusgalli seedling growth by effluents in the germination and early growth test

Concentration	Average weight (mg)		
%	Coke plant	Pulp mill	Waste plant
Darkness			
Control	0.4	0.4	0.6
1	0.4	0.4	0.4
10	0.4	0.3	0.3*
25	0.4	0.4	0.2*
50	0.3*	0.3*	0.3*
75	0.3*	0.2*	0.2*
100	0.2*	0.2*	0.2*
Light			
Control	1.2	1.2	1.5
1	1.0	1.5	1.3
10	0.8*	1.4	1.3
25	0.8*	1.4	1.0*
50	0.4*	1.1	0.3*
75	0.3*	0.8*	0.5*
100	0.1*	0.5*	0.3*

*, Significantly lower than control (P = 0.05).

Table 4. Dry weights (mg) of roots, shoots, and entire seedlings of Echinochloa crusgalli and Sesbania macrocarpa exposed to effluents in the hydroponic test

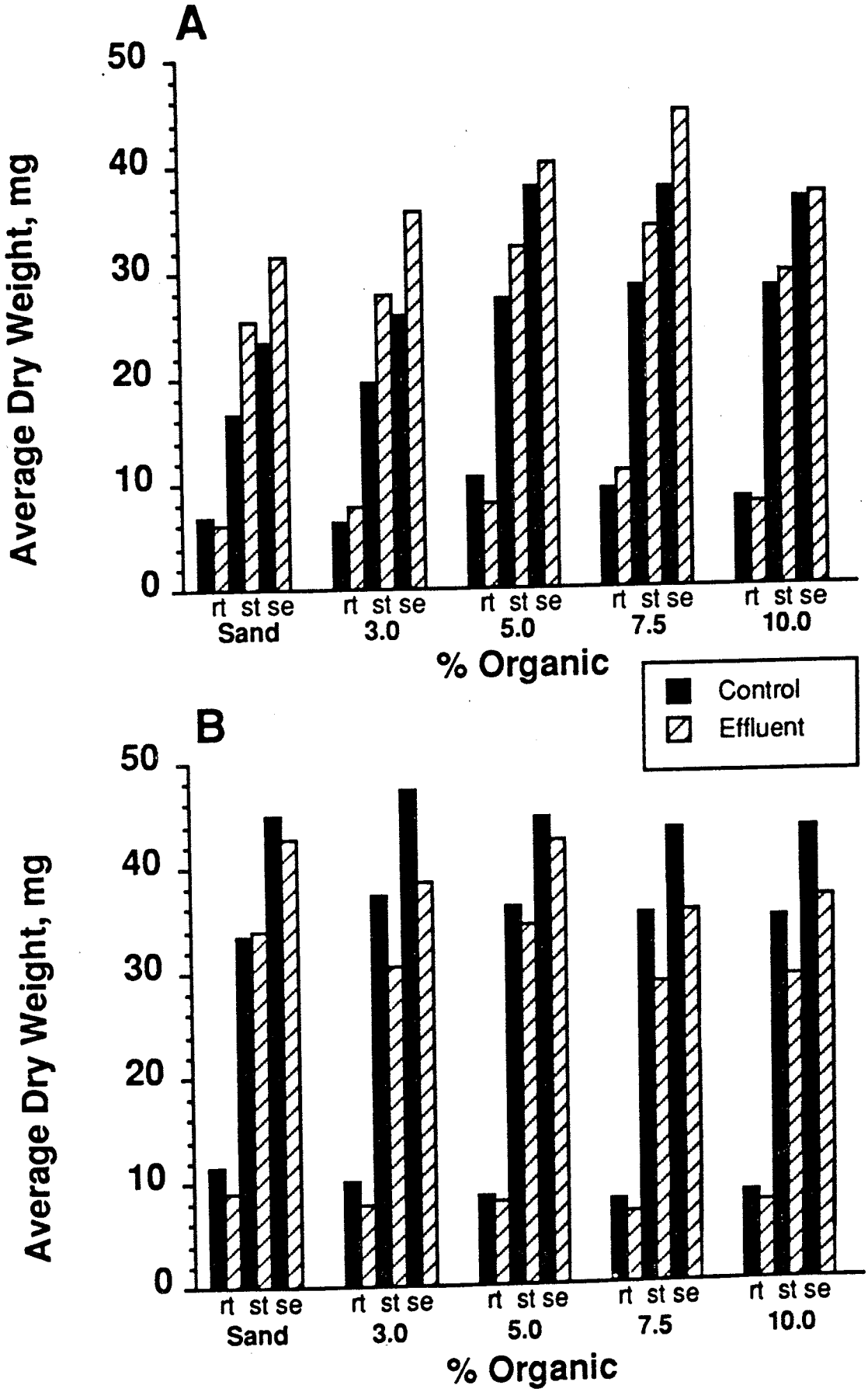
Concentration %	Coke plant		Pulp mill		Treatment plant	
	E. crusgalli	S. macrocarpa	E. crusgalli	S. macrocarpa	E. crusgalli	S. macrocarpa
Roots						
Control	19.3	11.9	20.2	21.4	25.7	14.8
1	22.3	7.7	9.4*	19.3	19.2*	14.0
10	13.8	7.5	2.6*	17.9	2.0*	12.5
25	5.6*	4.1*	1.8*	16.9	2.0*	8.5*
50	1.0	6.2*	2.4*	15.4	2.0*	10.7*
100	1.7*	2.2*	1.8*	16.2	1.4*	10.2*
Shoots						
Control	92.6	41.1	93.9	55.0	88.9	55.1
1	93.2	31.0	56.1*	49.4	64.2*	60.3
10	71.6	31.7	14.7*	50.7	11.1*	49.7
25	31.8*	17.4*	9.9*	37.3*	9.6*	40.5*
50	4.2*	22.6*	7.9*	35.7*	8.7*	35.4*
100	4.5	10.1*	6.7*	36.5*	6.4*	33.2*
Entire Seedlings						
Control	111.9	53.0	114.1	76.4	114.6	69.9
1	115.5	38.7	65.5*	68.7	83.4*	74.3
10	85.4	39.2	17.3*	68.6	13.1*	62.2
25	37.4*	21.5*	11.7*	54.2	11.6*	49.0*
50	5.2*	28.8*	10.3*	51.1	10.7*	46.1*
100	6.2*	12.3*	8.5*	52.7	7.8	43.4*

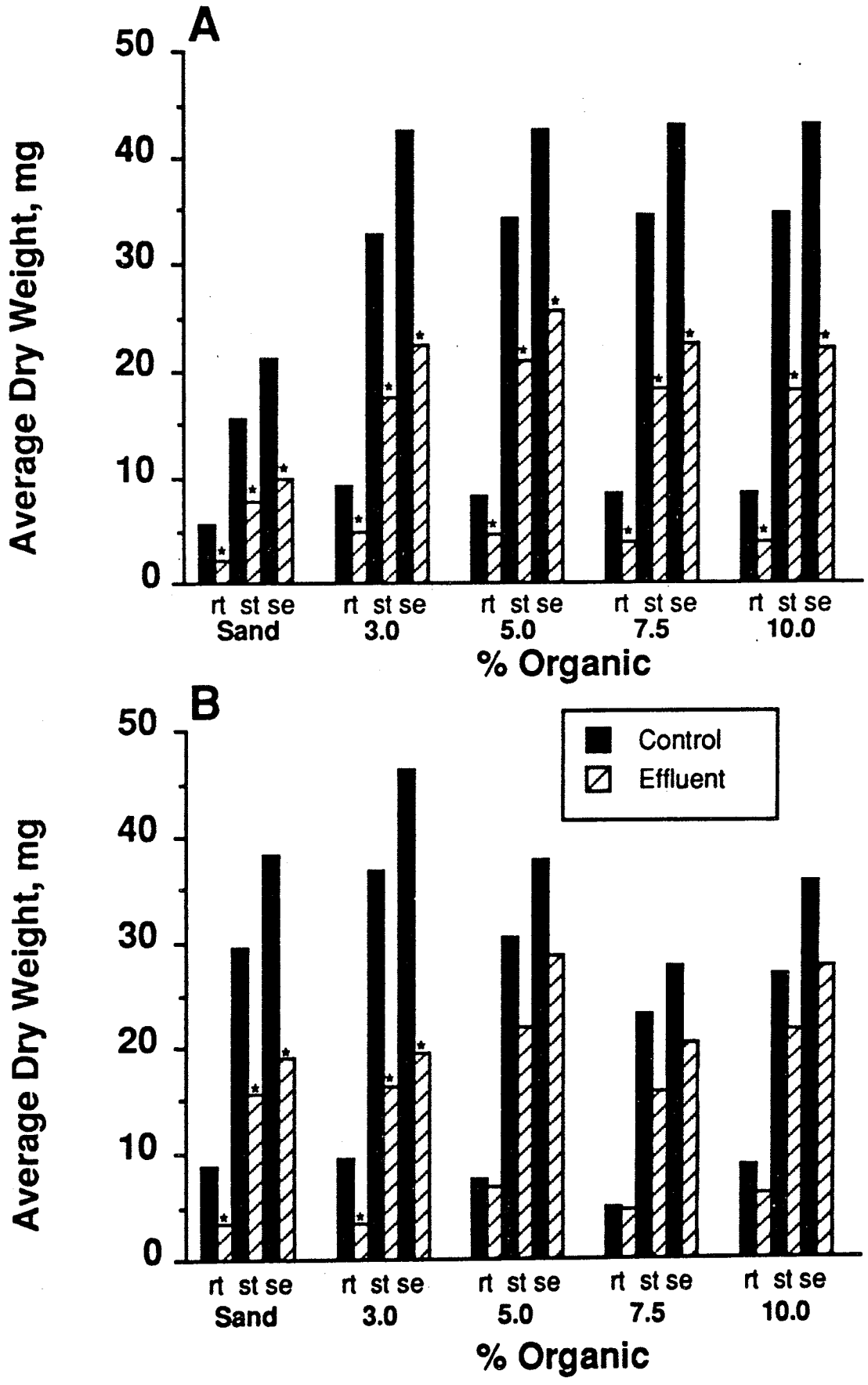
*, Significantly lower than control (P = 0.05)

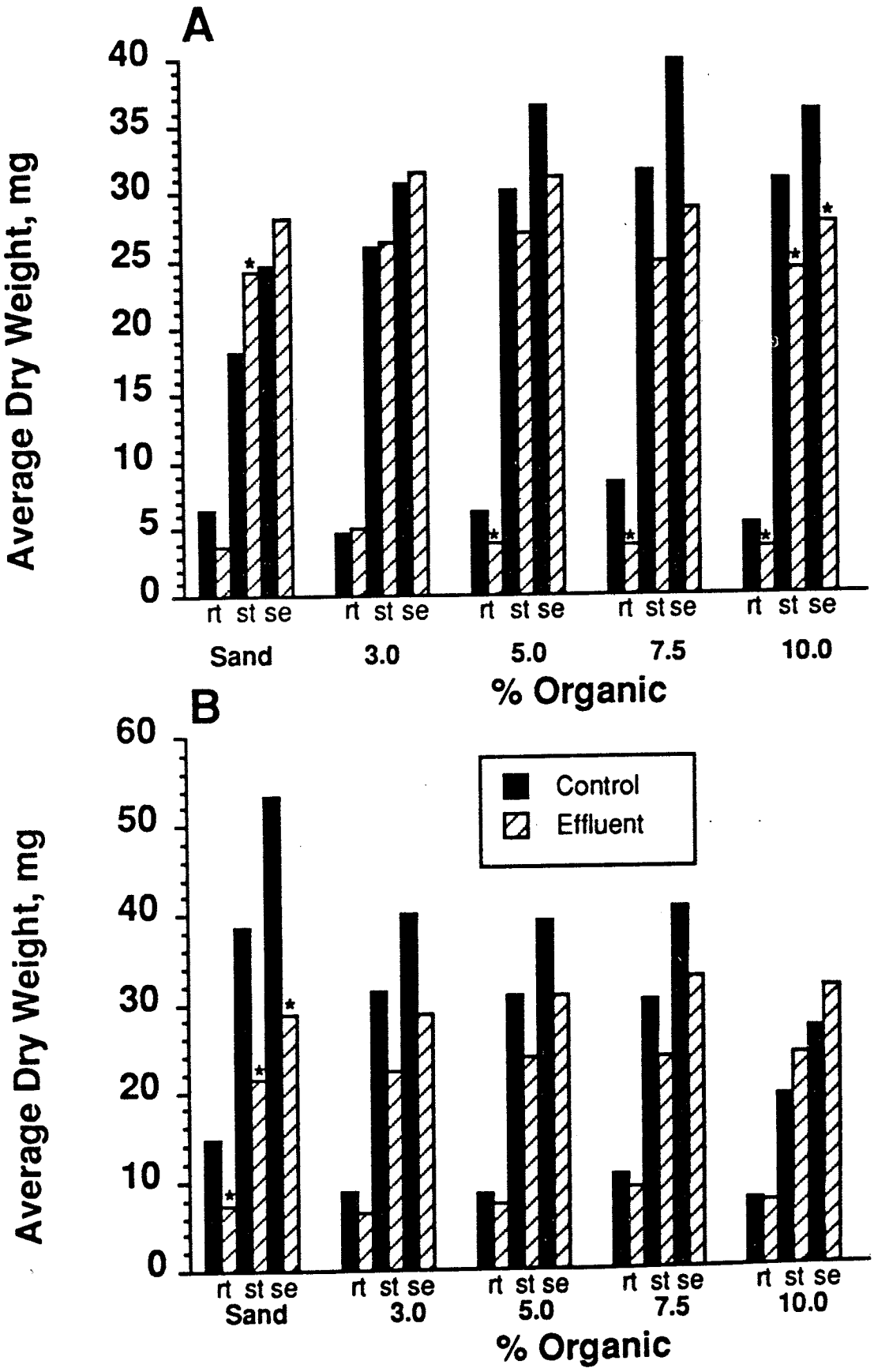
FIGURE TITLES

Figure

1. Average dry weights of Echinochloa crusgalli (A) and Sesbania macrocarpa (B) exposed to coke plant effluent in the sediment test. rt = roots, st = shoots, se = entire seedling.
2. Average dry weights of Echinochloa crusgalli (A) and Sesbania macrocarpa (B) exposed to pulp mill effluent in the sediment test. rt = roots, st = shoots, se = entire seedling.
3. Average dry weights of Echinochloa crusgalli (A) and Sesbania macrocarpa (B) exposed to wastewater treatment plant effluent in the sediment test. rt = roots, st = shoots, se = entire seedling.


A
Average Dry Weight, mg
50
40
30
20
10
0
rt st se
Sand
3.0
5.0
7.5
10.0
% Organic
Control
Effluent
B
Average Dry Weight, mg
% Organic


A
B
Average Dry Weight, mg
50
40
30
20
10
0
rt st se
Sand
3.0
5.0
7.5
10.0
% Organic
Control
Effluent


A
Average Dry Weight, mg
40
35
30
25
20
15
10
5
0
rt st se
Sand
3.0
5.0
7.5
10.0
% Organic
B
Average Dry Weight, mg
60
50
40
30
20
10
0
Control
Effluent
rt st se
Sand
3.0
5.0
7.5
10.0
% Organic

APPENDIX 4

ARTIFICIAL SEDIMENTS FOR USE IN TESTS WITH WETLAND PLANTS

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ARTIFICIAL SEDIMENTS FOR USE IN TESTS WITH WETLAND PLANTS

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WALSH G. E., WEBER D. E., BRASHERS L. K. and SIMON T. L. *Artificial sediments for use in tests with wetland plants*. ENVIRONMENTAL AND EXPERIMENTAL BOTANY **30,** 391–396, 1990.—Artificial sediments are described for use in studies on rooted marsh plants. The sediments, which are similar in particle size distribution to natural sediments, are formulated from commercially available sand, silt, clay and organic matter. Average survival rates of seedlings of *Echinochloa crusgalli* var. *crusgalli*, *Scirpus paludosus* and *Spartina alterniflora* were 93.4, 90.4 and 89.4%, respectively. Average seedling weight of each species was unaffected by percentage sand, silt, clay, organic matter, pH or CEC except for *S. paludosus*, which required organic matter in the sediment for maximal growth and whose growth was affected by the relationship between particle size and percentage sand.

INTRODUCTION

PARTICLE size distribution and organic content of sediments affect toxicity of organic and inorganic substances to rooted plants. In studies on effects of toxicants in sediments, a variety of sediment types is required that contain specific quantities of sand, silt, clay and organic matter. Natural sediments cannot provide the control required for the tests because factors of interest with regard to toxicity could not be isolated and varied experimentally. Also, natural sediments may be contaminated with toxicants or weed seeds. Consequently a method was devised by which a number of uncontaminated artificial sediments was formulated to provide the conditions required for growth of healthy plants.

Previous sediment and toxicity studies have employed unmodified natural sediments,[1,3,16] natural sediments amended with organic matter,[2] quartz sand,[20] and glass beads.[4] Artificial sediments composed of various amounts of sand, clay, calcium carbonate, and organic matter have also been used with earthworms,[13] fungi,[18] and bacteria.[4,7,9] An artificial system in which protein ions bound to glass microbeads was used as a substrate for nutritional analyses with marine deposit feeders.[17]

BRADSHAW[5] listed requirements for soil for growing plants. Such soil should have adequate bulk and particulate texture for rooting, adequate water retention properties, and be free of contaminants and weed seeds. This paper describes a method for formulation of artificial sediments from commercially available sand, silt, clay and particulate organic matter that satisfies these requirements. Survival and growth of two freshwater and one estuarine rooted plant species in artificial sediments that simulate natural sediments and 27 other artificial sediments are reported

Table 1. *Particle size distributions of natural sediments and simulated sediments used in survival and growth tests with* Echinochloa crusgalli, Scirpus paludosus *and* Spartina alterniflora

Class	Particle size (μm)	% Composition, by weight: Fresh water	Salt marsh
Coarse sand	500–1500	0.6	33.6
Medium sand	250–499	9.5	58.8
Fine sand	63–249	67.4	4.9
Silt	4–62	10.3	0.6
Clay	<4	6.7	0.7
Organic	—	4.9	0.8
Lost during analysis		0.6	0.6

here. Excellent survival and growth were obtained in the simulated and other artificial sediments.

METHODS

Natural sediment

Natural freshwater sediment was collected from a wetland area near Milton, FL; natural estuarine sediment was collected from a salt marsh at Garcon Point near Gulf Breeze, FL. Particle size distributions (Table 1) were determined by settling rate in water[6] and organic content by ashing.[12] Particle size distributions in the two natural sediments were simulated in formulation of artificial sediments.

Growth of plants in natural sediments is not reported because of the possibility of contamination by toxic chemicals. Also, natural sediments contained numerous weed seeds and their pHs declined with time in the plant growth chamber.

Artificial sediment

Dry silica sands were obtained from New England Silica, Inc., South Windsor, CT 06074-0185. They were "Mystic White"® No. 18 (coarse), No. 45 (medium), and No. 85 (fine). Data on particle size distributions and composition (Tables 2, 3) were obtained from the distributor. Each was composed of coarse, medium and fine grains, but was labelled according to the predominant particle size. The sands were washed thoroughly in tap and deionized water and dried in air before use. Dry silica clays (Attagel® and Attasorb® LVM) and silts (ASP® 400 and Attacote®) were obtained from Engelhard Corp., Edison, NJ 08818-2900. Data on particle sizes and composition (Tables 2, 3) were also provided by the distributor. Particulate organic matter was dried commercial peat humus (Green-

Table 2. *Particle size distributions of sands, and mean particle sizes of silts and clays used to formulate artificial sediments, from New England Silica Co. and Englehard Corp.*

Coarse mm	Coarse %	Medium mm	Medium %	Fine mm	Fine %
Sand					
0.42	2.5	0.15	0.4	0.05	Trace
0.60	10.0	0.21	6.2	0.07	4.5
0.84	37.5	0.30	48.4	0.11	20.1
1.19	42.5	0.42	40.7	0.15	32.3
1.68	7.5	0.60	4.1	0.21	33.4
		0.84	0.1	0.30	7.7
		Pan	0.1	0.42	0.5
				0.60	Trace
				Pan	1.5
Silt					
4.8 and 18 μm					
Clay					
0.1 and 2.0 μm					

Table 3. *Composition of sands, silts and clays used to formulate artificial sediments from New England Silica Co. (sands) and Engelhard Corp. (silts and clays)*

Sands (as oxides)	%	Silt and clay	%
SiO_2	97.7	SiO_2	65.9
Fe_2O_3	0.07	Fe_2O_3	3.6
Al_2O_3	1.5	Al_2O_3	12.2
Na_2O	0.01	TiO_2	0.5
TiO	0.04	CaCO	4.3
CaO	0.08	MgO	11.5
MgO	0.06	K_2O	0.8
K_2O	0.29	P_2O_5	1.1
Loss on ignition	0.25	Trace elements	0.1

Table 4. Values of pH and cation exchange capacity (CEC) for natural and artificial sediments used in survival and growth tests with Echinochloa crusgalli, Scirpus paludosus *and* Spartina alterniflora; *FW = fresh water, SM = salt marsh*

Artificial	CEC	pH FW	pH SM
0% *organic*			
25% coarse sand	92.4	8.8	8.3
25% medium sand	91.4	8.6	7.9
25% fine sand	84.8	8.6	8.0
50% coarse sand	57.0	8.7	8.1
50% medium sand	54.5	8.6	8.0
50% fine sand	43.9	8.7	8.1
75% coarse sand	24.2	8.9	8.2
75% medium sand	20.1	8.9	8.1
75% fine sand	19.4	8.9	8.2
1% *organic*			
25% coarse sand		8.6	8.0
25% medium sand		8.5	7.9
25% fine sand		8.5	8.0
50% coarse sand		8.5	8.0
50% medium sand	50.5	8.5	7.9
50% fine sand		8.6	8.0
75% coarse sand		8.6	8.1
75% medium sand		8.7	8.0
75% fine sand	—	8.7	8.1
10% *organic*			
25% coarse sand		7.8	7.4
25% medium sand		7.7	7.3
25% fine sand		7.7	7.4
50% coarse sand		7.6	7.3
50% medium sand	44.1	7.6	7.1
50% fine sand		7.5	7.3
75% coarse sand	—	7.2	7.1
75% medium sand	-	7.3	6.8
75% fine sand	—	7.6	7.3

leaf Products, Inc., Haines, FL 33844). It was milled to an average particle size of 840 μm on a Wiley mill.

The dry components were mixed in proportions similar to the natural sediments (Table 1) or common to other natural sediments [21] (Table 4). In the non-simulated artificial sediments, each grade of sand (coarse, medium, fine) was used in concentrations of 25, 50 and 75%. Organic concentrations were 0, 1 and 10%. Equal concentrations of silt and clay were adjusted according to sand and organic content. The dry sediments were reconstituted for growth studies by mixing with either deionized water or with natural sea-water diluted to 4 parts per thousand (ppt) salinity with deionized water at the ratio of 42 ml water : 135 g sediment. Sediments were mixed with a spatula in a large glass beaker until a smooth, homogenous mixture was obtained.

Approximately 100 ml of wet sediment was added to each of three styrofoam cups, 7.5 cm diameter × 5.5 cm high. Twenty milliliters of Hoagland solution [10] were added to each cup. At this time, the test system was comprised of a wet, compact sediment overlain by approximately 5 mm of nutrient solution, ready for introduction of marsh plant seedlings.

Sediment pH was determined by addition of 100 g sediment to 100 ml deionized water in a glass beaker. The mixture was stirred for 1 min and allowed to settle for 1 hr, at which time pH was determined with a Beckman Phi 12 pH/ISE meter. Sediment pH values are given in Table 4.

Cation exchange capacity (CEC) was determined for selected sediments (Table 4) by the ion-exchange analysis procedure. [19]

Marsh plants

Fresh water. Seeds of the common marsh plants, *Echinochloa crusgalli* var. *crusgalli* Palisot de Beauvois (barnyard grass, watergrass) and *Scirpus paludosus* A. Nels. (alkali bullrush), were obtained from Wildlife Nurseries, Oshkosh, WI 54903-2724. Taxonomic identities were confirmed by growing plants to maturity and using the nomenclature of HITCHCOCK [8] and HOTCHKISS. [11] Seeds were stored dry at 4°C. Three days before planting, seeds were surface sterilized by immersion in 1% sodium hypochlorite for 20 min, rinsed with deionized water, and allowed to germinate in deionized water in Petri dishes at 24±2°C. Light intensity during germination was approximately 35 $\mu E\ m^{-2}/sec^{-1}$ from cool white fluorescent tubes on a 16 hr light : 8 hr darkness cycle. Twelve 3-day old seedlings were planted in each cup and grown for 14 days under conditions identical to those for germination. Twenty milliliters of Hoagland solution was added 5 and 10 days after planting. Deionized water was added

as needed to keep a 5 mm deep layer of water over the sediment.

Salt water. Seeds of *Spartina alterniflora* were obtained from Environmental Concern, St. Michaels, MD 21663. They were stored in 4 ppt sea-water at 4°C. Six days before planting, the seeds were surface sterilized and rinsed as above and allowed to germinate in 4 ppt sea-water in Petri dishes under a daily temperature regime of 16 hr at 18°C and 8 hr at 35°C.[15] The lighting cycle and intensity were the same as for *E. crusgalli* and *S. paludosus*. Twelve 6-day old seedlings were planted in each cup and grown for 28 days under the conditions given for *E. crusgalli* and *S. paludosus*. Twenty milliliters of Hoagland solution was added to each cup 5, 10, 15 and 20 days after planting.

Measurement of dry biomass

At the end of the growth period, seedlings were harvested by peeling the styrofoam cup from the sediment and rinsing the sediment from the roots in deionized water in a large beaker. The sediment fell from the roots under gentle agitation and the entire plant was washed in a stream of deionized water. Seedlings from each cup were kept together, dried at 103°C for 24 hr, and weighed to within 0.1 mg.

Statistical analyses

Two methods were used for statistical analysis of survival and weight data. The average number of surviving seedlings and the average weight of seedlings per cup were calculated. Survival data were converted to logarithms. Comparisons of means in the overall test and between components of the artificial sediments were made with Tukey's Studentized Range Test.[14] Two-way analysis of variance (ANOVA) [14] was used to compare survival and growth in combinations of sand grain size, percentage sand, and percentage organic content ($\alpha = 0.05$).

RESULTS

Seedlings of the three species survived and grew well in all sediments (Table 5). Overall average survival was *E. crusgalli*, 93.4% (range in cups: 80.0–100%), *S. paludosus*, 90.4% (range in cups: 77.8–100%) and *S. alterniflora*, 89.4% (range in cups: 75.0–100%). There was no significant difference in survival among sediment groups for any species.

Average seedling weights of *E. crusgalli* and *S. alterniflora* were similar in all types of sediment (Table 5). Average seedling weight of *E. crusgalli* was 10.8 mg, and for *S. alterniflora*, average weight was 6.4 mg.

For *S. paludosus*, however, seedling weight was lower in the absence of organic matter in the sediment (Table 5). Average seedling weight in sediment without organic matter was 1.7 mg, whereas it was 2.4 mg in sediment with 1% organic content and 2.8 mg in sediment with 10% organic content. The difference was significant ($F = 16.63$, df = 2). Also, seedling weight of *S. paludosus* was influenced by the interaction of particle size and percentage sand (Table 6).

DISCUSSION

It is clear that particle size of sand, overall particle size distribution, percentage organic matter, pH, and CEC had no effect upon survival of *E. crusgalli*, *S. paludosus* and *S. alterniflora* seedlings when grown in single species culture.

Composition of artificial sediments had no effect on seedling weights of *E. crusgalli* and *S. alterniflora*. Particle size distribution, percentage sand, silt, clay and organic matter, and CEC varied widely, but seedling weights were never significantly different. Values of pH were approximately one unit lower in sediment with 10% organic content than in sediments with 0 and 1% organic content, but this had no effect on survival or seedling weight of these two species in single species culture. In contrast, growth of *S. paludosus* was significantly reduced by lack of organic matter.

However, seedlings of each species appeared normal in external morphology, and the data suggest that the species tested can survive and grow in monoculture under a wide range of substratum conditions.

It is also demonstrated that artificial sediments can be formulated from commercially available sands, clays, silts, and organic matter and that such sediments satisfy the requirements given by BRADSHAW.[5] Simulated sediments may be formulated in accordance with requirements of local

Table 5. Average number of seedlings that survived and average weight per seedling of Echinochloa crusgalli, Scirpus paludosus *and* Spartina alterniflora *grown in artificial sediments*

	E. crusgalli		*S. paludosus*		*S. alterniflora*	
	No. surviving	Av. weight (mg)	No. surviving	Av. weight (mg)	No. surviving	Av. weight (mg)
Simulated FW	34.0	11.2 (10.4–11.9)	33.0	2.7 (2.1–3.1)	—	—
Simulated SM	—	—	—	—	33.0	5.2 (4.4–5.9)
Sand type						
Coarse	32.5 (32–36)	10.8 (9.3–12.4)	31.3 (28–35)	2.6 (1.6–4.2)	31.3 (28–34)	6.4 (5.5–7.2)
Medium	34.4 (31–36)	10.3 (9.0–12.8)	33.6 (29–36)	2.2 (1.6–2.9)	32.7 (29–36)	6.7 (6.1–7.5)
Fine	33.8 (29–36)	11.2 (10.1–13.4)	33.0 (29–35)	2.1 (1.5–3.0)	34.2 (27–36)	6.6 (5.0–7.7)
% sand						
25	34.1 (32–36)	10.7 (9.6–13.3)	32.0 (29–35)	2.2 (1.6–3.5)	32.2 (29–36)	6.4 (5.6–7.4)
50	32.7 (31–34)	10.5 (9.0–12.8)	32.6 (29–36)	2.2 (1.6–2.9)	32.1 (28–35)	6.4 (5.5–7.7)
75	34.0 (29–36)	11.0 (9.2–12.4)	32.3 (28–35)	2.5 (1.7–4.2)	31.0 (27–34)	6.8 (6.7–7.6)
% organic						
0	33.9 (32–36)	10.4 (9.0–11.5)	33.4 (29–35)	1.7 (1.6–2.1)*	33.9 (30–36)	6.5 (5.0–7.7)
1	32.7 (29–36)	11.2 (9.7–12.8)	31.0 (29–36)	2.4 (1.8–4.2)	30.1 (27–33)	6.8 (5.8–7.6)
10	34.2 (32–36)	10.6 (9.1–12.4)	33.4 (30–36)	2.8 (2.1–3.9)	31.3 (29–34)	6.3 (5.5–7.5)

Thirty-six seedlings were planted in each group. Ranges are in parentheses. *, Statistically different from 1 and 10% organic ($\alpha = 0.05$). FW = fresh water; SM = salt marsh.

Table 6. Probability values from a two-way ANOVA of survival and average weight of seedlings of Echinochloa crusgalli, Scirpus paludosus *and* Spartina alterniflora *by combinations of sand type (coarse, medium, fine), percentage sand and percentage organic content*

	E. crusgalli		*S. paludosus*		*S. alterniflora*	
	No. surviving	Av. weight (mg)	No. surviving	Av. weight (mg)	No. surviving	Av. weight (mg)
Sand type vs % sand	0.7273	0.5014	0.5579	0.0347*	0.9903	0.4804
Sand type vs % organic	0.4104	0.1261	0.4014	0.4255	0.7992	0.1058
% Sand vs % organic	0.7676	0.4176	0.5713	0.4999	0.8535	0.3182

*, Significant interaction ($\alpha = 0.05$).

sedimentary conditions for studies on toxicity, survival, growth and nutritional requirements, competition, microbiology, and a variety of other edaphic factors. Important sediment conditions such as porosity, texture, bulk density, chemical composition (e.g. calcium-based instead of quartz-based components), or soil salinity can also be varied to suit individual requirements.

CONCLUSIONS

Artificial sediments that simulate natural sediment conditions of particle size and chemical composition can be formulated from commercially available sands, clays, silts, and organic matter. Such formulations have advantages over commercial potting mixes and other plant growth media because they allow design of well-defined sediments for specific purposes. They also do not have the disadvantages of natural sediments which may change drastically during a test, may contain toxic chemicals, weed seeds, or undesirable microbes and animals, and which may not be of desirable composition.

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Environmental Protection Agency, Environmental Research Laboratory, Gulf Breeze, FL, U.S.A.

Mention of trade names or commercial products in this report does not constitute endorsement by the U.S. Environmental Protection Agency.

REFERENCES

1. Barko J. W. and Smart R. M. (1979) The nutritional ecology of *Cyperus esculentus*, an emergent aquatic plant, grown on different sediments. *Aq. Bot.* **6,** 13–28.
2. Barko J. W. and Smart R. M. (1983) Effects of organic matter additions to sediment on the growth of aquatic plants. *J. Ecol.* **71,** 161–175.
3. Barko J. W. and Smart R. M. (1985) Laboratory culture of submersed freshwater macrophytes on natural sediments. *Aq. Bot.* **21,** 251–263.
4. Beveridge T. J., Meloche J. D., Fyfe W. S. and Murray R. G. E. (1983) Diagenesis of metals chemically complexed to bacteria: laboratory formulation of metal phosphates, sulfides, and organic condensates in artificial sediments. *Appl. envir. Microbiol.* **45,** 1094–1108.
5. Bradshaw A. D. (1989) The quality of topsoil. *Soil Use Mgmt* **5,** 101–108.
6. Folk R. L. (1980) *Petrology of sedimentary rocks*. Hemphill, Austin, TX.
7. Gile J. D. and Gillett J. W. (1979) Fate of selected fungicides in a terrestrial laboratory ecosystem. *Ag. Food Chem.* **27,** 1159–1164.
8. Hitchcock A. S. (1950) *Manual of the grasses of the United States*. 2nd Edn. U.S. Govt Printing Office, Washington, DC.
9. Ho W. C. and Ko W. H. (1986) Microbiostasis by nutrient deficiency shown in natural and synthetic soils. *J. gen. Microbiol.* **132,** 2807–2815.
10. Hoagland D. R. and Arnon D. I. (1950) *The water culture method for growing plants without soil*. Circular 347. California Experiment Station, Berkeley, CA.
11. Hotchkiss N. (1972) *Common marsh, underwater and floating-leaved plants of the United States and Canada*. Dover Publications, New York.
12. Jackson M. L. (1960) *Soil chemical analyses*. Prentice Hall, Englewood Cliffs, NJ.
13. Neuhauser E. F., Loehr R. C. and Malecki M. R. (1986) Contact and artificial soil tests using earthworms to evaluate the impact of wastes in soil. Pages 192–203 *in* J. K. Petros, Jr, W. J. Lacey and R. A. Conway, eds *Hazardous and industrial solid waste testing: Fourth Symposium*. American Society for Testing and Materials, Philadelphia, PA.
14. SAS Institute, Inc. (1982) *SAS User's Guide: Statistics*. 1982 Edn. SAS Institute, Inc., Cary, NC.
15. Seneca E. D. (1974) Germination and seedling response of Atlantic and Gulf Coasts populations of *Spartina alterniflora*. *Am. J. Bot.* **61,** 947–956.
16. Smart R. M. and Barko J. W. (1980) Nitrogen nutrition and salinity tolerance of *Distichlis spicata* and *Spartina alterniflora*. *Ecology* **61,** 630–638.
17. Taghon G. L. and Jumars P. A. (1984) Variable ingestion rate and its role in optimal foraging behavior of marine deposit feeders. *Ecology* **65,** 549–558.
18. Titus J. A. and Pfister R. M. (1982) Effects of pH, temperature, and Eh on the uptake of calcium by bacteria and an artificial sediment. *Bull. Envir. Contam. Toxicol.* **28,** 697–704.
19. USDA (1972) *Soil survey laboratory methods and procedures for collecting soil samples*. Soil Survey Investigations Report No. 1. Soil Conservation Service, Washington, DC.
20. USFDA (1987) *Environmental assessment technical handbook*. U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition and Center for Veterinary Medicine, Environmental Impact Staff, Washington, DC.
21. Wild A. (ed.) (1988) *Russell's soil conditions and plant growth*, 11th Edn. John Wiley and Sons, New York.

APPENDIX 5

SYNTHETIC SUBSTRATA FOR PROPAGATION AND TESTING OF SOIL AND SEDIMENT ORGANISMS

USEPA, Gulf Breeze, U.S.A.

Synthetic substrata for propagation and testing of soil and sediment organisms[1]

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1. Introduction

Studies on soil- or sediment-dependent species must often be conducted in the laboratory. Laboratory studies may be confounded by the fact that similar natural test substrata are difficult to obtain when taken at different times or from different localities. A variety of artificial substrata has been proposed to reduce variability between tests, and laboratory studies on soil- and sediment-dependent organisms have been conducted with substrata that were not similar to natural ones (USDA, 1972; BEVERIDGE et al., 1983; TAGHON & JUMARS, 1984; ANTON et al., 1990) or with standard substrata designed to be similar to natural ones (TITUS & PFISTER, 1982; BEVERIDGE et al., 1983; HEIMBACH, 1985; HO & KO, 1986; NEUHAUSER et al., 1986;

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VAN GESTEL et al., 1989; ANTON et al., 1990). Data from test substrata that are not similar to natural substrata are suspect with regard to extrapolation to field conditions (SPIES, 1989; VAN STRAALEN & DENNEMAN, 1989), and standard substrata may be too simple in composition to relate adequately to natural substrata.

However, growth of seedlings of freshwater and estuarine plants in sediments designed to be similar to natural sediments with various amounts of sand, clay, and silt was as rapid or more rapid than growth in natural sediments (Walsh et al., 1990). Also, responses of freshwater and estuarine plants to toxicants were similar in natural and synthetic sediments (Walsh et al., 1991 a,b). Synthetic sediments were less likely to change during the course of experiments. For example, pH tended to decline and, in the case of natural estuarine sediments, reached such low values (<2.5) that toxicants were inactivated. It was clear from these comparative studies that synthetic sediments could be formulated to yield high survivorship and normal growth of plants when compared to natural sediments without undesirable change.

Our studies with a variety of aquatic and terrestrial plants and animals required a method whereby sediments and soils could be formulated for specific experimental requirements. These requirements were: the various substrata must be composed of the same organic and inorganic constituents, but in different relative amounts; they must be capable of being formulated to exact specifications; and their physical and chemical characteristics must be similar to those of natural soils and sediments.

A method is described here for formulation of soils and sediments used in tests with aquatic and terrestrial vascular plants, earthworms, and freshwater and marine crustaceans and vertebrates. The method was developed to (1) employ commercially available silicate sand, silt, clay, and particulate and dissolved organic matter for formulation of substrata with any desired particle size distribution and organic content, and (2) to ensure that such synthetic substrata were similar to

natural soils and sediments with regard to pH, Eh, cation exchange capacity (CEC), conductivity, and extractable acids and bases.

2. Materials and methods

2.1 Composition of substrata

The basic formulation was: 850 g washed medium quartz sand ("Mystic White" No. 18, New England Silica, Inc., South Windsor, CT), 150 g of a clay and silt mixture (ASP 400, Englehard Corp., Edison, NJ), 22 g sphagnum moss (Hyde Park Products, Inc., Mamaroneck, NY), 0.1 g soluble humic acids (Aldrich Chem. Co., Milwaukee, WI), and 0.5 g dolomitic limestone (Southern Agri-Minerals Corp., Hartford, AL). This formulation contained 3% organic matter. A commercial mixture of cow manure and organic compost (Hyponex Corp., Atlanta, GA) was added to the basic formulation to increase the organic content: 21 g for 5%, 80 g for 7.5%, and 145 g for 10% organic content. Methods for hydration of the dry substratum mixture are described for each of the test species. After hydration, the substrata were allowed to equilibrate for 48 h, after which time pH, Eh, and CEC were constant.

Sphagnum moss and the cow manure/organic compost mix were rinsed for removal of clay and silt before use. Sand was washed thoroughly in deionized water. Sphagnum and manure/compost mix were soaked for 5 days in deionized water, with daily replacement of water, to remove acids. They were dried at 60°C and milled to an average particle size of 840 μm on a Wiley mill.

2.2. Physical and chemical characteristics

Chemical and physical characteristics of the four formulations after 48 h of equilibration are given in Table 1. Redox potential (Eh) of the synthetic sediments was measured with a Radiometer/Copenhagen PHM 80 pH/Eh meter fitted with a platinum electrode, and pH with a

Beckman Phi 12 pH meter (JACKSON, 1960). Other methods were: particle size distribution (FOLK, 1980), total organic matter (JACKSON, 1960), and CEC, conductivity, extractable acids,, and extractable bases (USDA, 1972).

2.3 Submersed plant

The submersed freshwater macrophyte, Egeria densa Planch, was collected from a pond near Pensacola, Florida. It was maintained in deionized water for 1 week in the laboratory and appeared healthy and disease-free. The species was tested for survival and growth in sand, the clay/silt mix, and the four synthetic sediments according to the method of SMART & BARKO (1980). Five hundred grams of each substratum were added to each of three 4-L glass jars. Three stems of E. densa were planted in each jar and the jars filled with hard freshwater (SMART & BARKO, 1980). Each stem was 10 cm long and roots were not present. The plants were placed under 110 μEm^{-2} sec^{-1} light from cool white fluorescent tubes with a 16 h light: 8 h darkness cycle at 25 ± 1°C and allowed to grow for 3 weeks. Water was replaced after 1 and 2 weeks of incubation. At the end of the growth period, plants were collected and their lengths measured. Where branches had grown from the original plant, their lengths were added to yield total length. Roots were separated from stems, dried at 103° for 24 h, and weighed.

2.4 Wetland plants

Seeds of the freshwater wetland plants, Echinochloa crusgalli (L.) Palisot de Beauvois var. crusgalli, and var. zelayensis, and Sesbania macrocarpa Muhl., were obtained from Wildlife Nurseries, Oshkosh, WI. Seeds of the saltwater marsh plant, Spartina alterniflora Loisel, were obtained from Environmental Concern, Inc., St. Michaels, MD. The freshwater species were stored dry at 4°C and the saltwater species was stored 4°C in filtered seawater diluted to $4°/_{oo}$ salinity.

Before planting, the seeds were treated to remove possible fungal contaminants and to break dormancy. Seeds of E. crusgalli and S. alterniflora were soaked in 1% sodium hypochlorite solution for 20 min and rinsed with deionized water. Seeds of S. macrocarpa were immersed in concentrated sulfuric acid for 30 min, rinsed, and soaked overnight in deionized water.

Sediments were prepared by adding 126 ml of Hoagland solution (HOAGLAND & ARNON, 1950) to 405 g dry sediment. Hoagland solutions were made with deionized water for tests with freshwater species and with 4°/$_{oo}$ diluted seawater for tests with S. alterniflora. Approximately 100 ml of wet sediment were placed in each of 3 styrofoam cups, 5.5 cm high x 7.5 cm diameter. This yielded a wet system in which the sediment was overlain by approximately 5 mm of nutrient solution.

Seedlings cultivated in washed coarse sand under light and temperature conditions given above were planted in the sediments 24 h after preparation. Ten seedlings of both E. crusgalli varieties (5 d old) and S. alterniflora (7 d old) and 3 seedlings of S. macrocarpa (7 d old) were planted in each sediment, and each exposure was done in triplicate. Seedlings were incubated for 2 weeks under the conditions given above. They were watered with nutrient solution each Monday, Wednesday, and Friday. After 2 weeks, survival was recorded, the seedlings were removed carefully from the substrata, rinsed, dried at 103°C for 24 h and weighed.

2.5 Terrestrial plants

Four species of terrestrial plants were tested with the four synthetic soils and a commercial seedling starter mix (Premier Pro Mix®, Premier Branch, Inc., New Rochelle, NY), a mixture of peat and pearlite. The species were: Lactuca sativa L. ("Early Butterhead" bib lettuce) and Daucua carota L. ("Scarlet Nantes" carrot) from Northrup King Co., Minneapolis, MN, Tagetes erecta L.

("Mellow Yellow" marigold) from Ferry-Morse Seed Co., Fulton, KY, and Trifolium incarnatum L. Funks G Hybrid (Crimson clover), Dothan Seed and Supply Co., Inc., Dothan, AL.

Seeds were treated with 1% sodium hypochlorite, and seedlings were grown as described above for 7 days before planting. The substrata were lightly wetted with Hoagland nutrient solution and placed in small plastic pots with drainage holes. One seedling of clover and carrot was placed in each of 12 pots; one seedling of lettuce and marigold was planted in each of 5 pots. The seedlings were grown for 24 d under lighting, temperature, and watering conditions given above. At harvest, seedlings were removed carefully from the soils, dried and weighed as above.

2.6 Earthworms

The earthworm, Eisenia foetida (Savigny), was tested for survival and reproduction in the four synthetic organic sediments and in sphagnum peat moss, a standard earthworm substratum. Earthworms were from the culture of the U.S. Environmental Protection Agency, Environmental Research Laboratory, Corvallis, OR. They were cultured in sphagnum peat moss at the Gulf Breeze Laboratory in darkness at 25°C for 2 months before use. During that time, the worms reproduced and appeared healthy.

Ten individuals of E. foetida were added to duplicate 3.6 L glass containers that contained one L of moss wetted with deionized water or each wetted synthetic soil. The containers were covered with a loose-fitting wooden top to minimize water loss by evaporation and to allow air exchange. Cultures were maintained in a moist environment by addition of deionized water to the substrata twice each week. Nutrition was provided by addition of 25 g of partially decomposed rabbit food (Wayne Feed Co., Washington Park, IL) to the surface of the substrata once each week for the duration of the test. The cultures were maintained in darkness at 25 ± 1°C. After 2 months, adult, young, and cocoons were picked from the substrata and counted.

2.7 Crustaceans

Survival and growth tests were conducted with synthetic sediments in freshwater with the red crayfish, Procambarus clarki (Girard), and in saltwater with the mysid, Mysidopsis bahia Molenock, and the grass shrimp, Palaemonetes pugio Holthuis. Juveniles of the crayfish were obtained from M-K Ranches, Wewahitchka, FL; mysids from the Gulf Breeze laboratory culture.

Crayfish were exposed to the synthetic sediments in plastic food storage containers, 12.5 cm high and 10 cm square. Control crayfish were kept in boxes without sediment. Each container, except controls, received 405 g of sediments, to which was added 300 ml of dechlorinated water, yielding a depth of approximately 8 cm. There were six replicates of each sediment type and the control. Each crayfish was weighed and its length measured, and one was placed in each container with a sprig of E. densa. The animals were maintained at 25 ± 1° under 35 μEm^{-2} sec^{-1} light from cool white fluorescent tubes on a 16 h light: 8 h darkness cycle. Water was drained and replaced and molts and survival recorded each day for 4 weeks. Crayfish were fed one pellet of M-K Ranch Crawfish Pellets each Monday, Wednesday, and Friday. After 4 weeks, the crayfish were weighed and their lengths measured.

Two static tests were conducted with mysids and sediment extract. They were standard toxicity tests as conducted for analysis of sediments (USEPA, 1978). Seven hundred g of each synthetic sediment were mixed into 2.8 L of natural filtered seawater diluted to $20°/_{oo}$ salinity with deionized water. The supernatant liquid was removed and used for testing after a 24 h period of settling. Dilutions were made with $20°/_{oo}$ salinity filtered seawater and the pH adjusted to between 7.7 and 8.1 with 10N NaOH. After adjustment of pH, 800 ml of test liquid were added to each of three culture dishes and 10 juvenile mysids were added randomly to each dish. Thus, 30 mysids were exposed to extracts from each synthetic sediment. An equal number of mysids was exposed in the same way to $20°/_{oo}$ natural seawater and to extract from washed silica sand as controls. Exposure

was for 96 h, as in the standard acute mysid test, and for 7 days, as in the standard chronic test. Both tests were conducted at 20 ± 1°C under a 14 h light: 10 h darkness cycle. The mysids were fed 24-h-old Artemia sp. nauplii each day. Survival of mysids at the end of each test is reported.

Grass shrimp were maintained in flow-through aquaria without sediment, with washed silica sand, and with the four synthetic sediments. In this system (SPRAGUE, 1969; WARD & PARRISH, 1980), exposure was in screened cups with 170 g of sand or sediment on the bottom. Cups were placed in aquaria fitted with automatic siphons that allowed filling with filtered salinity-controlled seawater through the screen to a depth of approximately 8 cm, with subsequent drainage of water to a depth of approximately 3 cm. Flow rate was 12.6 1 h^{-1}. Five grass shrimp from the Gulf Breeze Laboratory brood stock were placed randomly in each cup, and each control, sand, and sediment exposure was done in duplicate. Salinity of the water was 20°/$_{oo}$ and the temperature was 25 ± 2°C. Approximately 1400 24-day old A. salina nauplii were added as food to each cup every day during the 38-day test. At that time, surviving shrimp were harvested, dried for 24 h, and weighed.

2.8 Vertebrates

Two vertebrate species were tested with the synthetic sediments: Bufo valliceps, Wiegmann (Gulf Coast Toad, freshwater) and Cyprinodon variegatus (sheepshead minnow, saltwater). Toads were collected as tadpoles from a pond near Pensacola, FL. Washed silica sand and the four synthetic sediments were added to Carolina culture dishes, 10 cm i.d. and 5 cm tall. Dechlorinated water was added to a depth of 4.5 cm and a 10 cm long branch of E. densa and 6 tadpoles were placed randomly in each dish. Tadpoles were fed as much Tetramin® fish food as they could consume in 15 min and observed for survival each day. Metamorphosed individuals were removed

when their tails were no longer present. Water in each dish was replaced each week. Tests were terminated after 40 days, when all tadpoles had metamorphosed into young toads.

The system for exposure of C. variegatus was identical to that for P. pugio. Ten fish larvae, less than 24-h-old with an average length of 2.8 mm, were placed randomly in cups that contained the synthetic sediments and observed for survival each day for 38 days. At that time, fish were dried at 103°C for 24 h and weighed.

2.9 Statistical analysis

Data were evaluated statistically by a general linear model for analysis of variance (ANOVA). When F values of the ANOVA were significant (P = 0.05), means of groups were compared by Duncan's Multiple Range Test (SAS, 1989).

3. Results

3.1 Submerged plants

All plants survived in this test. Production of E. densa was poor in sand, but growth was rapid in the synthetic organic sediments (Table 2). Average increase in length, total number of branches produced, and average dry weight of roots were significantly greater in the sediments than in sand. The total number of shoots produced was the same in all sediments, but the average increase in length was greater in 5, 7.5, and 10% organic content than in 3%, and average root weight in sediment was inversely related to organic content.

3.2 Wetland plants

There was 100% survival of all species of wetland plants in all synthetic sediments, and there was no effect of organic matter on average weights of E. crusgalli zelayensis or S. alterniflora. Growth of E. crusgalli crusgalli was greatest in 7.5% organic sediments; growth of S. macrocarpa was greater in 5, 7.5 and 10% organic sediment than in 3% (Table 3).

3.3 Terrestrial plants

There was 100% survival of clover, carrot, and lettuce in all soils. In the synthetic sediments, 100% of the marigolds survived, but 3 survived and 2 died in the commercial mix.

Growth of all species in sediments was equal to or greater than that in the commercial seedling starter mix (Table 4). Growth of clover and carrot was similar in all substrata. Compared to the starter mix, there was significantly greater growth of lettuce in 7.5% and marigold in 5% organic sediments.

3.4 Earthworm

Of the 100 adult earthworms in sphagnum and synthetic sediments, only one died (in 5% organic sediment). Eisenia foetida reproduced in all substrata (Table 5). Reproduction was greatest in sphagnum and synthetic sediments with 7.5 and 10% organic content. Combined production of cocoons was significantly lower in soils with 3 and 5% organic content than in the other synthetic substrata.

3.5 Crustaceans

There was 100% survival of P. clarki in each group except synthetic sediment with 5% organic content, where 1 animal died (Table 6). Also, there were no significant differences in the number

of molts, average gain in length, or average gain in weight among the control and sediment groups (Table 6).

There was no effect of extracts of sand and sediments on survival of M. bahia when compared with the seawater control in acute and chronic tests (Table 7). Although there appeared to be a trend toward diminished numbers with increasing organic content in the chronic test, the differences were not statistically significant.

Of 60 P. pugio used in this test, only one died in the synthetic sediment with 7.5% organic content (Table 8). There was no effect of sand or sediment on average length or average weight of the shrimp (Table 8).

3.6 Vertebrates

Tadpoles of B. valliceps developed normally and there was no relationship between rate of metamorphosis and composition of the substratum (Table 9). All tadpoles metamorphosed by Day 40.

There was also no effect of sand or sediment on survival and average length of C. variegatus (Table 10). However, average dry weights of the young fishes in sand and sediments were significantly greater than those of fishes maintained in the absence of substratum (Table 10).

4. Discussion

The results above demonstrate that synthetic soils and sediments of various compositions support survival and growth of a wide variety of plants and animals. Although use of a single reference soil (EDWARDS, 1983; OECD, 1984) is of value for standardization of test methods, it may also be important to formulate specific substrata for specific needs, such as requirements of

individual species (WALSH et al., 1990) or for toxicity tests with substrata of specific compositions (WALSH et al., 1991, a,b).

Synthetic substrata offer advantages over natural substrata for testing with plants and animals (Table 11). Besides careful control of structure (particle and pore sizes), other aspects such as pH, Eh, CEC, etc. can be controlled by judicious use of $CaCO_3$, type of sand (siliceous vs calcareous), and type of clay or silt (e.g. montmorillonite, kaolinite, illite, vermiculite, etc.) in various relative concentrations. Such formulations may also be used for amendment of natural soils in laboratory and field studies.

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6. Literature cited

ANTON, F., E. LABORDA, & P. LABORDA, 1990. Acute toxicity of the fungicide captan to the earthworm Eisenia foetida (Savigny). Bull. Environ. Contam. Toxicol. **45**, 82-87.

BEVERIDGE, T.J., J.D. MELOCHE, W.S. FYFE, & R.G.E. MURRAY, 1983. Diagenesis of metals chemically complexed to bacteria: laboratory formulation of metal phosphates, sulfides, and organic condensates in artificial sediments. Appl. Environ. Microbiol. **45**, 1094-1108.

EDWARDS, C.A., 1983. Development of a standard laboratory method for assessing the toxicity of chemical substances to earthworms. Commission of the European Communities, Director-ate-General Environmental, Consumer Protection and Nuclear Safety, EUR8714EN, Brussels.

FOLK, R.L., 1980. Petrology of sedimentary rocks. Hemphill, Austin, TX.

HEIMBACH, F., 1985. Comparison of laboratory methods, using Eisenia foetida and Lumbricus terrestris, for the assessment of the hazard of chemicals to earthworms. Zeit. Pflanzenkh. Pflanzensch. **92**, 189-193.

HOAGLAND, D.R., & D.I. ARNON, 1950. The water culture method for growing plants without soil. Circular 347, California Experiment Station, Berkeley.

HO, W.C., & W.H. KO, 1986. Microbiostasis by nutrient deficiency in natural and synthetic soils. J. gen. Microbiol. **132**, 2807-2815.

JACKSON, M.L. 1960. Soil chemical analysis. Prentice Hall, Englewood Cliffs, NJ.

NEUHAUSER, E.F., R.C. LOEHR, & M.R. MALECKI, 1986. Contact and artificial soil tests using earthworms to evaluate the impact of wastes in soil. pp. 192-203. In: PETROS, J.K. JR., W.J. LACEY, & R.A. CONWAY (eds.), Hazardous and industrial solid waste testing: fourth symposium, American Society for Testing and Materials, Philadelphia.

OECD, 1984. OECD guidelines for testing of chemicals. Test 207: Earthworm acute toxicity tests. Organization for Economic Cooperation and Development, Paris.

SAS Institute Inc., 1989 SAS/STAT User's Guide Version 6, 4th ed., Vol. 1. SAS Institute Inc., Cary, NC.

SMART, R.M. & J.W. BARKO, 1980. Nitrogen nutrition and salinity tolerance of Distechlis spicata and Spartina alterniflora. Ecology **61**, 630-638.

SPIES, R.B. 1989. Sediment bioassays, chemical contaminants and benthic ecology: new insights or just muddy water? Mar. Environ. Res. **27**, 73-75.

SPRAGUE, J.B., 1969. Measurement of pollutant toxicity to fish. I. Bioassay methods for acute toxicity. Water Res. **3**, 793-821.

TAGHON, G.L., & P.A. JUMARS, 1984. Variable ingestion rate and its role in optimal foraging behavior of marine deposit feeders. Ecology **65**, 549-558.

TITUS, J.A., & R.M. PFISTER, 1982. Effects of pH, temperature, and Eh on the uptake of calcium by bacteria and an artificial sediment. Bull. Environ. Contam. Toxicol. **28**, 697-704.

USDA, 1972. Soil survey laboratory methods and procedures for collecting soil samples, Soil Survey Investigations Report No. 1. Soil Conservation Service, Washington, DC.

USEPA, 1978. Bioassay procedures for the ocean disposal permit program. U.S. Environmental Protection Agency, EPA-600/9-78-010, Gulf Breeze, FL.

VAN GESTEL, C.A.M., W.A. VAN DIS, E.M. VAN BREEMEN, & P.M. SPARENBURG, 1989. Development of a standardized reproduction toxicity test with the earthworm species Eisenia foetida andrei using copper, pentachlorophenol, and 2,4-dichloroaniline. Ecotox. Environ. Safety **18**, 305-312.

VAN STRAALEN, N.M., & C.A. DENNEMAN, 1989. Ecotoxicological evaluation of soil quality criteria. Ecotox. Environ. Safety. **18**, 241-251.

WALSH, G.E., D.E. WEBER, L.K. BRASHERS & T.L. SIMON, 1990. Artificial sediments for use in tests with wetland plants. Environ. Exp. Bot. **30**, 391-396.

WALSH, G.E., D.E. WEBER, T.L. SIMON, & L.K. BRASHERS, 1991a. Toxicity tests of effluents with marsh plants in water and sediment. Environ. Toxicol. Chem. **10**, 517-525.

WALSH, G.E., D.E. WEBER, T.L. SIMON, L.K. BRASHERS, & J.C. MOORE, 1991b. Use of marsh plants for toxicity testing of water and sediment. In: GORSUCH J., & W. WANG. Use of plants for toxicity assessment, STP 1091, American Society for Testing and Materials, Philadelphia in press.

WARD, G.S. & P.R. PARRISH, 1980. Evaluation of early life stage toxicity tests with embryos and juveniles of sheepshead minnows. pp. 243-247. In: EATON, J.G., P.R. PARRISH, & A.C. HENDRICKS (eds.), Aquatic toxicology, STP 707, American Society for Testing and Materials, Philadelphia.

Synopsis: Original scientific paper

WALSH, G.E., D. WEBER, L. ESRY, M. NGUYEN, J. NOLES, & B. ALBRECHT, 1991. Synthetic substrata for propagation and testing of soil and sediment organisms. Pedobiologia

A method for formulation of synthetic substrata (soils and sediments) is given. Submersed, wetland, and terrestrial plants, earthworms, crustaceans, and vertebrates were maintained on synthetic substrata composed of various amounts of commercially available sand, clay, silt, and particulate and dissolved organic matter. Total organic matter of the synthetic substrata were 3, 5, 7.5, and 10% by weight. All test species survived and grew well in the substrata. It is suggested that synthetic substrata have advantages over natural substrata in laboratory tests with plants and animals. Among the advantages are: synthetic sediments may be formulated for specific studies, differences in texture and chemical characteristics between batches are minimized, and the substrata are not contaminated by anthropogenic substances as are many natural soils and sediments.

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Table 1. Characterization of synthetic sediments used in toxicity tests with wetland plants after 48 h equilibration.

Substratum total organic matter (%)	Percent sand (mm) 1-2	.5-1	.25-.5	.1-.25	.05-.1	Silt (mm) .002-.05	Clay (mm) <.002
3	0	13.4	63.8	5.6	0.6	12.1	4.5
5	0	13.8	62.0	5.4	0.6	13.2	5.0
7.5	0.2	13.8	58.0	5.8	0.6	16.1	5.5
10	0	12.8	56.0	6.6	0.8	20.0	3.8

	Cond. (mS/cm)	Extract. bases (meq/100g)	Extract. acids (meq/100g)	pH	Eh	CEC (meq/100g)
3	0.17	4.86	0.16	6.4	462	5.1
5	0.37	7.77	0.52	6.8	412	8.3
7.5	0.83	17.16	1.74	6.9	373	18.9
10	1.33	25.96	2.07	7.2	347	28.0

Table 2. Average increase in length, number of branches produced, and dry weight of roots of Egeria densa after 3 weeks of growth in sand and synthetic sediments. Values with different letters are significantly different from each other, P = 0.05.

Total organic matter (%)	Average increase in length, (cm)	Total number of branches	Average root weight (g)
0 (sand)	4.4^{A}	15^{A}	0^{A}
3	19.7^{B}	32^{B}	34.7^{B}
5	31.5^{C}	31^{B}	26.9^{C}
7.5	32.8^{C}	28^{B}	17.0^{D}
10	26.6^{C}	34^{B}	11.4^{E}

Table 3. Average seedling weights (mg) of wetland plants grown for 2 weeks in synthetic sediments. Values with different letters within each taxon are significantly different from each other, P = 0.05.

	Total organic matter (%)			
	3	5	7.5	10
E. crusgalli	8.8^{A}	9.8^{A}	11.1^{B}	9.7^{A}
E. crusgalli zelayensis	35.6^{A}	38.8^{A}	31.3^{A}	38.2^{A}
S. macrocarpa	15.9^{A}	21.2^{B}	21.2^{B}	19.9^{B}
S. alterniflora	5.9^{A}	4.7^{A}	4.6^{A}	4.4^{A}

Table 4. Average seedling weights (mg) of terrestrial plants grown for 2 weeks in a commercial seedling starter mix and synthetic sediments. Values for each species with different letters are significantly different from each other, P = 0.05.

	Commercial	Total organic matter (%)			
	mix	3	5	7.5	10
Clover	29.8^{A}	32.6^{A}	30.5^{A}	30.7^{A}	35.9^{B}
Carrot	14.3^{A}	10.2^{A}	11.9^{A}	13.5^{A}	13.7^{A}
Lettuce	54.2^{A}	61.7^{A}	79.3^{A}	98.3^{B}	64.9^{A}
Marigold	39.9^{A}	40.6^{A}	46.6^{B}	37.0^{A}	31.5^{A}

Table 5. Survival and reproduction of Eisenia foetida for 2 months in sphagnum peat moss and synthetic sediments. Twenty adults were in each group at the start of the test. Values with different letters are significantly different from each other, P = 0.05.

Substratum total organic matter (%)	Surviving adults	Young worms	Cocoons	Total young and cocoons
90.1 (sphagnum)	20^{A}	$135^{A,B}$	149^{A}	284^{A}
3	20^{A}	62^{B}	59^{B}	121^{B}
5	19^{A}	$125^{A,B}$	36^{B}	161^{B}
7.5	20^{A}	306^{A}	122^{A}	428^{A}
10	20^{A}	232^{A}	172^{A}	404^{A}

Table 6. Survival, number of molts, and growth of Procambarus clarki for 4 weeks on synthetic sediments. Six crayfish were in each group at the beginning of the test. Differences between the control and synthetic sediments are not significant, P = 0.05.

Total organic matter (%)	Number of survivors	Number of molts	Av. gain in length (mm)	Av. gain in weight (g)
0 (control)	6	11	9.9	0.71
3	6	10	12.7	0.93
5	5	9	12.6	1.03
7.5	6	12	13.3	1.00
10	6	11	11.3	0.86

Table 7. Survival of Mysidopsis bahia in extracts of sand and synthetic sediments in acute (96 h) and chronic (7 d) tests. Thirty animals were in each group at the beginning of the test.

Total organic matter (%)	Number survived	
	acute	chronic
0 (seawater control)	29	30
0 (sand)	29	29
3	29	29
5	28	28
7.5	30	26
10	29	25

Table 8. Survival and dry weights of Palaemonetes pugio exposed to sand and synthetic sediments for 38 d in a flow-through system. Ten animals were in each group at the beginning of the test. Differences between groups were not significant, P = 0.05.

Total organic matter (%)	Number of survivors	Average length (mm)	Average dry weight (mg)
0 (no substrate)	15	22.3	198
0 (sand)	15	22.0	195
3	15	22.9	195
5	15	22.0	207
7.5	14	23.2	191
10	15	22.5	191

Table 9. Cumulative metamorphosis of Bufo valliceps on sand and synthetic sediments. Six toads were in each group.

	Number metamorphosed					
Total organic	Days on sediment					
matter (%)	33	35	36	38	39	40
0 (sand)			1	2	5	6
3	1			2	4	6
5		1		4	6	
7.5				2	4	6
10			1	3		6

Table 10. Survival, standard length, and dry weight of Cyprinodon variegatus on sand and synthetic sediments for 38 d. Twenty fishes were in each group. Values with different letters are significantly different from each other, P = 0.05.

Total organic matter (%)	Number of survivors	Average length (mm)	Average dry weight (mg)
0 (no substrate)	20^{A}	14.9^{A}	23.1^{A}
0 (sand)	19^{A}	15.4^{A}	28.3^{B}
3	19^{A}	15.7^{A}	30.6^{B}
5	20^{A}	15.7^{A}	29.8^{B}
7.5	19^{A}	15.5^{A}	28.9^{B}
10	19^{A}	15.6^{A}	29.4^{B}

Table 11. Advantages and disadvantages of natural and synthetic substrata in tests with plants and animals.

Natural substrata
Advantages
Contain all of the constituents found in natural systems (particulate, dissolved, nutrients, organisms, etc.)
Inexpensive
Preparation is not time-consuming
Disadvantages
Cannot be formulated as desired
Inconsistent composition among batches collected at different times
Change physically and chemically with storage and drying
Must be stored at low constant temperature
May be contaminated
Contain organisms (e.g. invertebrates, weed seeds) that can confound results; methods to remove organisms may alter substrata
Synthetic substrata
Advantages
Support desirable survival and growth rates of plants and animals
Can be formulated as desired for texture, chemical composition, etc.
Can be prepared as needed, with little difference in texture and chemical composition between batches

Are not contaminated

Can be stored dry at room temperature

pH can be stabilized

Do not contain organisms other than incidental bacteria

Disadvantages

May not contain all of the constituents of natural substrata

Components must be purchased

Preparation is relatively time-consuming

APPENDIX 6

SYNTHETIC SEDIMENTS: A TOOL FOR RESEARCH

SYNTHETIC SEDIMENTS: A TOOL FOR RESEARCH

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ABSTRACT

Formulation of synthetic sediments for use in ecological and toxicity testing is described. Results of toxicity tests with complex effluents in synthetic sediments that contained various concentrations of organic matter are compared with results from hydroponic tests. It is shown that the sediment system affects toxicity of complex effluents and it is suggested that both sediment and hydroponic tests be performed for rigorous risk assessment.

INTRODUCTION

Laboratory testing with sediments often requires rigorous control of physical and chemical characteristics such as particle size distribution, pH, Eh, cation exchange capacity, etc. Although natural sediments contain all of the constituents of natural systems, they have some disadvantages in research areas such as aquatic ecology and toxicity testing. They cannot be formulated to specific experimental requirements, there is inconsistent composition among samples collected at different times (Stemmer et al., 1990), they undergo chemical and physical changes when stored, and they may be contaminated with chemicals or undesired organisms (Bradshaw, 1989; Walsh et al., in press a).

Synthetic sediments offer an alternative to natural sediments when physical and chemical conditions must be carefully controlled. They can be formulated as desired with commercially available sand, clay, silt, and dissolved and particulate organic matter, and they support survival and growth of submersed, wetland, and terrestrial plants as well as freshwater and estuarine animals (Walsh et al., in press b). In addition, they can be prepared as needed, with little difference among lots, are not contaminated, can be stored dry at room temperature, and do not contain undesirable organisms.

This report describes formulation and use of synthetic sediments in toxicity tests with wetland plants and complex effluents.

Responses to effluents in sand and in sediments of different organic contents are compared with responses in hydroponic culture to demonstrate effects of sediment systems on toxicity.

MATERIALS AND METHODS

Synthetic Sediments

Preparation of synthetic sediments has been described in detail (Walsh et al., in press a, b). Their compositions are given in Table 1. They were prepared by mixing 135 g of the dry constituents (Table 1) into 42 ml of plant nutrient solution (Hoagland and Arnon, 1950) or effluent that contained the same nutrients. Some characteristics of this wet sediment are given in Table 2.

Effluents

Effluents tested were from chemical and sewage treatment plants. After collection by grab sampling, they were packed in ice and shipped in insulated chests to the U.S. Environmental Protection Agency, Gulf Breeze, Florida. Upon receipt within 24 h, they were checked for pH, salinity, color, suspended solids, and odor. They were stored in darkness at 4 C and used in toxicity tests the day after receipt.

Test Species

The test species was Echinochloa crusgalli (L.) Beauv. var crusgalli. Echinochloa crusgalli is a monocot widely distributed in freshwater wetlands and terrestrial habitats of the U.S. Seeds were obtained from Wildlife Nurseries, Oshkosh, WI.

Hydroponic Survival and Growth Test

The hydroponic test was conducted to establish toxicity of effluents in direct contact with roots in liquid. Five days before the test began, seeds were planted in coarse sand wetted with deionized water and incubated at 25 ± 1 C under 110 $\mu E/m^2/s$ cool white fluorescent light. On the day of the test, seedlings were harvested, their bases wrapped with cotton, and the roots immersed in nutrient solution prepared with deionized water (control) or effluent dilutions, to which nutrients were added in a 125 ml Erlenmeyer flask. The cotton plug prevented the seedling from falling into the medium and suppressed evaporation of medium. Six seedlings were exposed to control and effluent media under the same temperature and lighting conditions as above. Seedlings were harvested after a total of two weeks exposure. At harvest, the seedlings were divided into roots and shoots, dried for 24 h at 103 C, and weighed.

Table 1. Compositions of synthetic sediments used in toxicity tests with Echinochloa crusgalli. 0.01 g of humic acids was added to each synthetic sediment.

Components	Composition, % by Weight			
	3% Organic	5% Organic	7.5% Organic	10% Organic
Sand	82.8	81.1	76.7	72.5
Clay and silt	14.6	14.3	13.6	12.8
Dolomite	0.5	0.5	0.5	0.4
Sphagnum moss	2.1	2.1	2.0	1.9
Cow manure/compost	-	2.0	7.2	12.4

Suppliers

Medium sand:	Mystic White No. 45, New England Silica, Inc. South Windsor, CN
Clay and silt:	ASP 400, Englehard Corp., Edison, NJ
Dolomite:	Southern Agri-Minerals Corp., Hartford, AL
Sphagnum moss:	Hyde Park Products, Inc., Mamroneck, NY
Cow manure/compost:	Hyponox Corp., Atlanta, GA
Humic acids:	Aldrich Chemical Co., Milwaukee, WI

Table 2. Characteristics of synthetic sediments used in toxicity tests with wetland plants.

Substratum (% organic)	Percent Sand (mm)					Silt (mm)	Clay (mm)
	1-2	.5-1	.25-.5	.1-.25	.05-.1	.002-.05	<.002
3	0	13.4	63.8	5.6	0.6	12.1	4.5
5	0	13.8	62.0	5.4	0.6	13.2	5.0
7.5	0.2	13.8	58.0	5.8	0.6	16.1	5.5
10	0	12.8	56.0	6.6	0.8	20.0	3.8

	Extract. bases (meq/100g)	Extract. acids (meq/100g)	pH	Eh	CEC (meq/100g)	Cond. (mmho/cm)
3	4.86	0.16	6.4	462	5.1	0.17
5	7.77	0.52	6.8	412	8.3	0.37
7.5	17.16	1.74	6.9	373	18.9	0.83
10	25.96	2.07	7.2	347	28.0	1.33

Sediment Survival and Growth Test

Sediment toxicity tests were conducted to evaluate any effect sediment may have on toxicity of the effluents, compared to effect in liquid medium. Ten seedlings, grown as above, were planted in each of three styrofoam cups, 5.5 cm high x 7.5 cm diameter, that contained sediment prepared as above. Similar cups were prepared with washed medium sand as a control. The seedlings were grown for 2 weeks under the conditions described. At harvest, shoots and roots were dried, and weighed as above.

Statistical Analysis

Differences between treatment means were evaluated by analysis of variance (SAS 1989). Treatment means were compared with the control by Duncan's multiple range procedure when F values were significant ($P = 0.05$; SAS, 1989).

RESULTS AND DISCUSSION

Chemical Plant Effluent

There was no effect of chemical plant effluent on survival of *E. crusgalli* in the hydroponic or sediment tests.

Hydroponic test, growth. Seedling weights were affected by chemical plant effluent (Fig. 1). Although not statistically significant, there appears to be a trend toward greater average shoot and entire seedling (root and shoot) weights in 1, 10, and 25% effluent when compared with the control. (See the "sediment" section below, where weights of seedlings treated with effluent in sand were greater than those of untreated seedlings.) Conversely, in 50 and 100% effluent, average weights of roots ($P = 0.0014$), shoots ($P = 0.0003$), and entire seedlings ($P = 0.0002$) were significantly lower than average weights of the controls.

Sediment test, growth. The effluent caused significant increase in average weights of shoots ($P = 0.0021$) and entire seedlings ($P = 0.0094$) in sand (Fig 2). Here average weight of shoots exposed to the effluent was 56.1% greater than that of the control group; average weight of entire seedlings exposed to the effluent in sand was 50.0% greater than that of the control. These data, with the apparent trend toward enhanced growth of seedlings treated with low concentrations of effluent in the hydroponic test, suggest that plant nutrients were present in the sample tested. Although the effluent was toxic in the hydroponic test, it was not in the sediment test.

Sewage Treatment Plant Effluent

Sewage treatment plant effluent did not affect survival of *E. crusgalli* in the hydroponic and sediment tests.

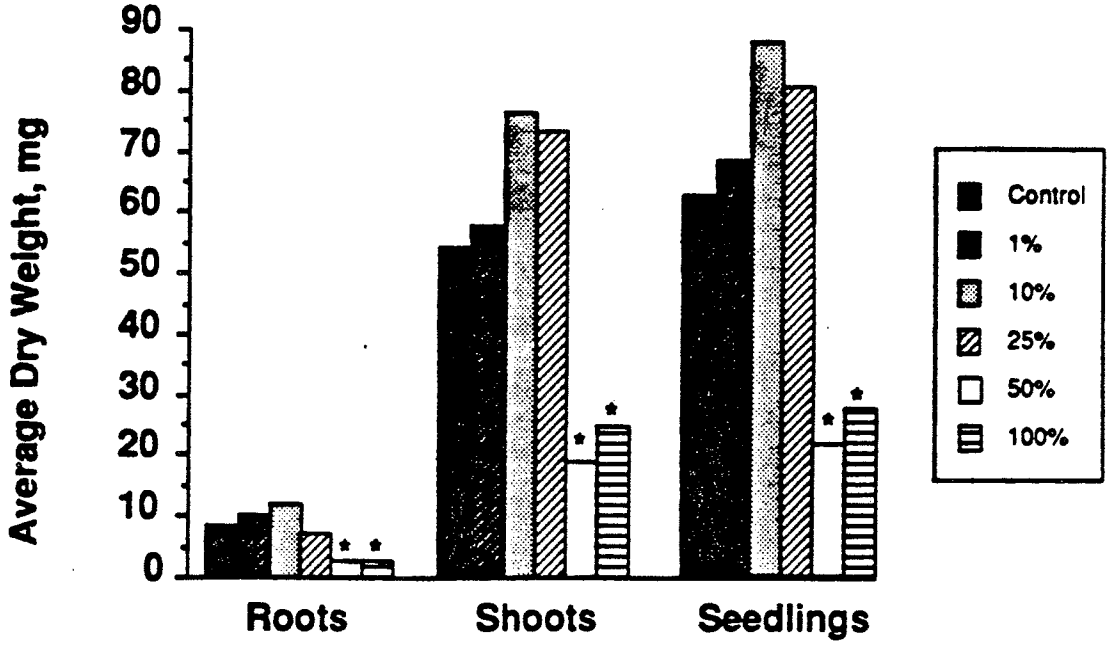

Fig. 1. Average dry weights of roots, shoots, and entire seedlings of Echinochloa crusgalli exposed to chemical plant effluent in the hydroponic test. * = significantly lower than control, P = 0.05.

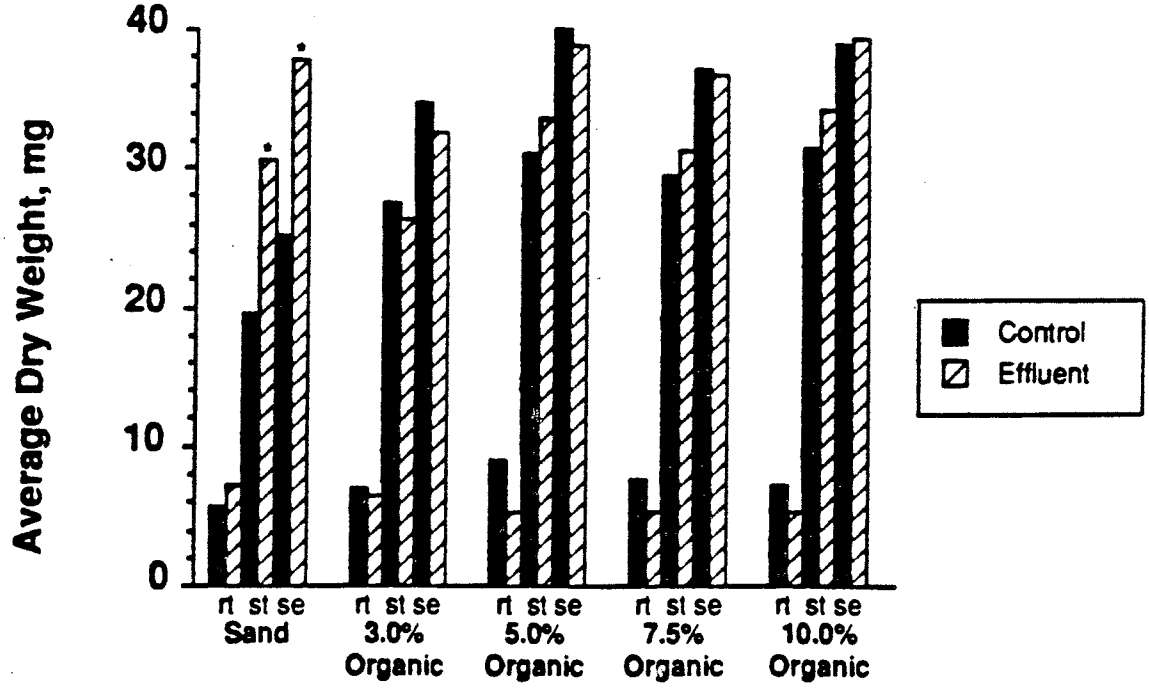

Fig. 2. Average dry weights of roots (rt), shoots (st), and entire seedlings (se) of Echinochloa crusgalli exposed to chemical plant effluent in sand and synthetic organic sediments. * = significantly greater than control, P = 0.05.

Hydroponic test, growth. The effluent inhibited growth of E. crusgalli in the hydroponic test (Fig. 3). Average root weight was significantly lower than that of the control in concentrations of 10% effluent and greater (P = 0.0001), and average shoot (P = 0.001) and average entire seedling (P = 0.0001) weights were significantly lower than the controls at 25% and above effluent.

Sediment test, growth. The effluent significantly inhibited growth of E. crusgalli in sand, but not in the synthetic sediments (Fig. 4). In sand, average weight of treated roots was 20.3% of the control (P = 0.0098), average weight of treated shoots was 38.7% of the control (P = 0.0005) and average weight of entire seedlings was 32.8% of the control (P = 0.0066).

These results demonstrate that sediment can reduce toxicity of complex effluents to wetland plants. Both effluents were toxic to E. crusgalli in the hydroponic test but not in sediments that contained as little as 3% organic matter. Stimulation of growth by chemical plant effluent as in the hydroponic test and sand did not occur in the organic sediments.

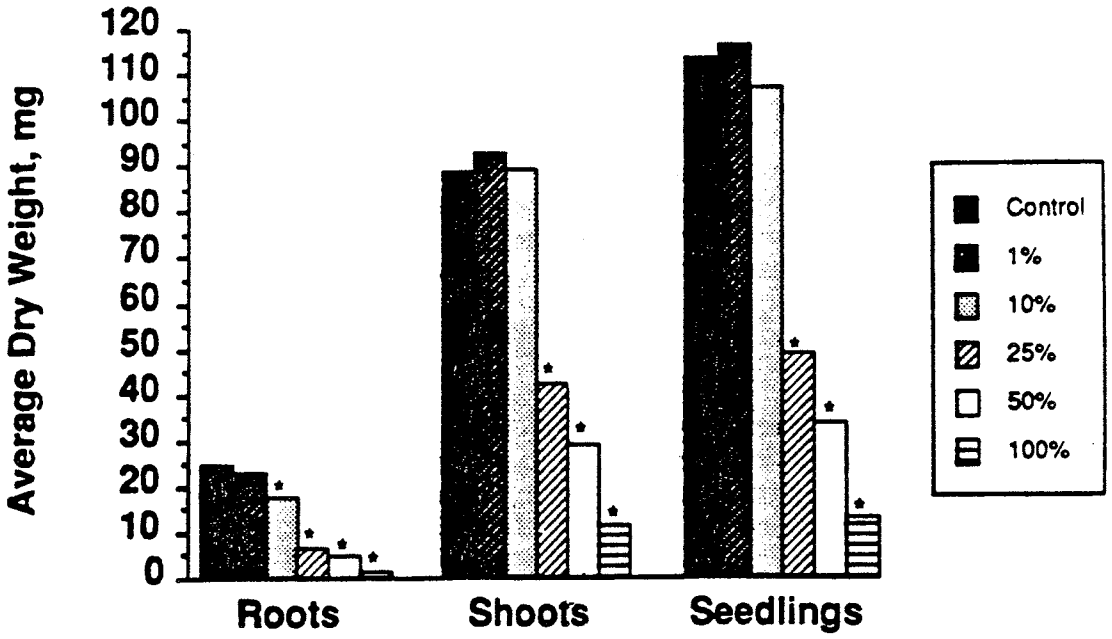

Fig. 3. Average dry weights of roots, shoots, and entire seedlings of Echinochloa crusgalli exposed to sewage treatment plant effluent in the hydroponic test. * = significantly lower than control, P = 0.05.

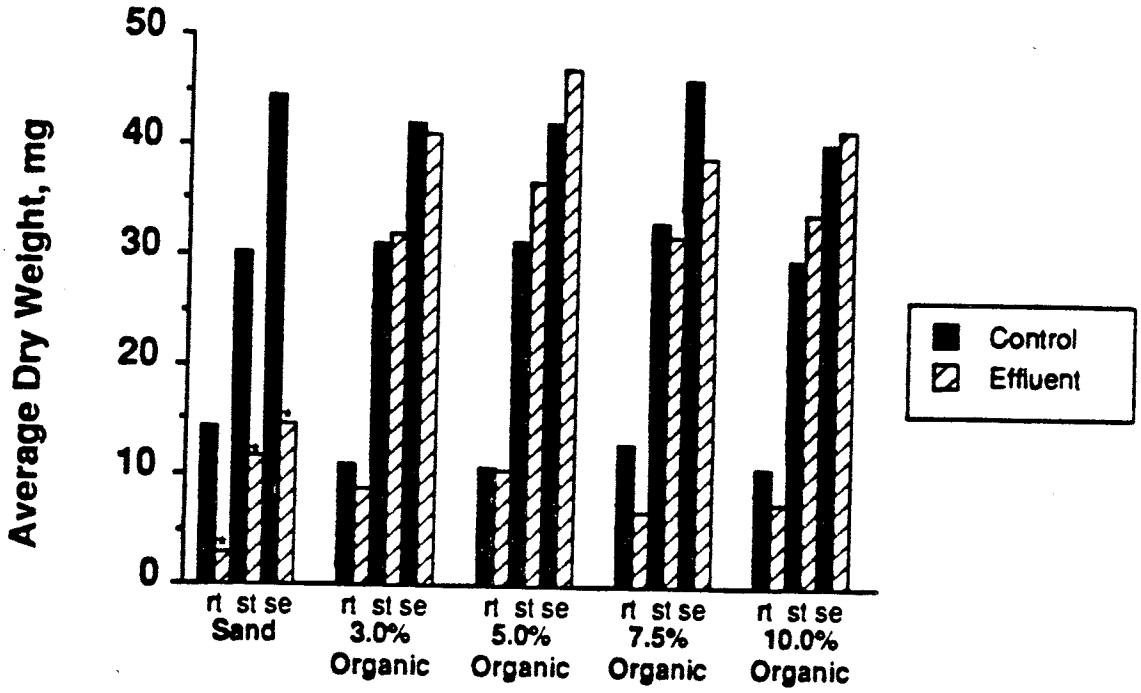

Fig. 4. Average dry weights of roots (rt), shoots (st), and entire seedlings (se) of Echinochloa crusgalli exposed to sewage treatment plant effluent in sand and synthetic organic sediments. * = significantly lower than control, P = 0.05.

CONCLUSION

It is concluded that hydroponic tests can identify phytotoxic effluents but sediments may affect toxicity of some effluents to plants. For a complete risk assessment, it is suggested that hydroponic and sediment tests be performed, one to show potential effects of the untreated effluent, and the other to provide a more realistic estimate of possible effects in the field.

LITERATURE CITED

Bradshaw, A.D. 1989. The quality of topsoil. Soil Use Mgmt. 5: 101-108.

Hoagland, D.R. and D.I. Arnon. 1950. The Water Culture Method for Growing Plants Without Soil. California Agricultural Experiment Station, Circular 347, Berkeley, CA.

SAS Institute, Inc. 1989. SAS/STAT User's Guide. Version 6, 4th ed., Vol. 1, SAS Institute, Inc., Cary NC.

Stemmer, B.L., G.A. Burton, Jr., and G. Sasson-Brickson. 1990. Effect of sediment spacial variance and collection method on cladoceran toxicity and indigenous microbial activity determinations. Environ. Toxicol. Chem. 9:1035-1044.

Walsh, G.E., D.E. Weber, T.L. Simon, and L.K. Brashers. Toxicity tests of effluents with marsh plants in water and sediment. in.: J. Gorsuch and W. Wang, eds. Use of Plants for Toxicity Assessment, STP 1091, American Society for Testing and Materials, Philadelphia, PA. in press a.

Walsh, G.E., D.E. Weber, L.K. Esry, M.T. Nguyen, J. Noles, and B. Albrecht. Synthetic substrata for propagation and testing of soil and sediment organisms. Biol. Fertil. Soils. in press b.

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